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SECTION MEETINGS

MINNESOTA	
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PEIPING	
Peiping Union Medical College	March 17, 1939

10548 P

Agents Causing Cardiac Supernormality.

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Studies have been conducted on the electrical excitability of the excised turtle ventricle perfused by means of a Straub type cannula with an oxygenated, bicarbonate buffered saline mixture. The preparations have in some cases not been spontaneously rhythmic or have beat at a slow rate. On these it has been possible to obtain rather complete data concerning the changes in excitability throughout the cardiac cycle. In other instances with spontaneously rhythmic, rapidly beating ventricles, it has been possible to obtain only limited information.

Supernormality has been produced or increased by increase in the ratio of calcium to potassium in the perfusate, by reduction of the sodium bicarbonate, by addition of veratrin, and by addition of

strophanthin. The period of supernormality has in many cases been followed by a period of prolonged subnormality.

In the nonrhythmic preparation, the development of supernormality may result in the production of 2 or more responses following a single electrical stimulation. Such Lucianni groups have been directly attributed to supernormality by Ashman and Hafkesbring.[1] In the spontaneously rhythmic preparation, supernormality may result in the production of occasional extrasystoles, of regular coupling of beats, and of shorter or longer bursts of tachycardia. In this stage there may be multiple beat foci and with severe poisoning fibrillation may ensue. The similarity of this series of events to the effects seen with toxic dosages of the digitalis-like drugs on the mammalian ventricle is striking and there seems no reason to doubt that the mechanism is the same for the two cases.

The methods by which we have produced supernormality in the ventricular preparation are those which will cause its production in the nerve trunk (Graham[2, 3]). Moreover, the subnormal period (Graham[4]) may be found in the two preparations following supernormal period (see also Ashman and Wooley[5]). The results of these experiments, thus offer further indication of the similarity in the two preparations, of the fundamental processes concerned with the recovery of electrical excitation following a response despite the very great differences in their manifest time functions.

10549 P

Relation of Muscle Receptive Substance to the Contractile Mechanism. Responses to Heat and Drugs.

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This paper reports further work in an investigation of muscle-receptive substance and contractile mechanism in smooth muscle

1 Ashman, R., and Hafkesbring, R., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 162.

2 Graham, H. T., *Am. J. Physiol.*, 1933, **104**, 216.

3 Graham, H. T., *Am. J. Physiol.*, 1934, **110**, 225.

4 Graham, H. T., *Am. J. Physiol.*, 1935, **111**, 452.

5 Ashman, R., and Wooley, E., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 159.

fibers, already reported briefly.[1, 2, 3] The results here reported seem to confirm the hypothesis that "This receptive substance is very labile to heat."[2] They seem to prove that the receptive substance can be destroyed without "killing" the contractile mechanism. The results, therefore, suggest that the receptive substance is not, as Langley[4] postulated, a side chain of the contractile mechanism, but rather an independent structure or substance as postulated by Dixon.[5]

Method. The muscles here studied were the iris muscles of fetal pigs about 5 days before birth (length, 260 mm, plus or minus 10 mm). The fetuses were obtained from the recently killed mother, quickly excised, measured, and the marked heads placed in a mixture of hog saline and saline ice. For testing, the eyes of 7 fetuses of a litter were excised, placed in small individual pyrex glass cups in pairs, and covered with a glass plate. One eye of each pair was used as a control, the other for the test. The action of a drug or of a temperature change was measured by measuring the short diameter of the pupil before and after application. This measurement was made with fine bow dividers under illumination by both transmitted and reflected light, and the value read off on a diagonal scale. This value was corrected for any simultaneous change in the control.

To determine the lability of the receptive substance to heat of 40°C, 6 of the 7 pairs of eyes were placed in a thermoregulated physiological saline bath at 40°C and a pair removed at regular intervals and cooled in a mixutre of saline and saline ice. The unheated pair of eyes was used as a control in the subsequent tests. The destruction of the cholinergic receptive substance was measured by the amount of decrease in pupil constriction to 3 drops of 0.3% carbaminoyl choline hydrochloride dropped onto the cornea. The tonus in the dilator muscle fibers was measured by the amount of dilation after atropinizing the carbaminoyl choline treated muscles with 2 drops of 0.01% atropine sulfate. To test the irritability and contractility of the contractile mechanism after destruction of the receptive substance, the eyes were rapidly cooled or heated, and the amount of pupillary constriction or dilation measured.

Results. The facts here reported were derived from a study of

1 Shaklee, A. O., and Christensen, K., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1619.

2 Shaklee, A. O., Christensen, K., and Oppenheimer, H. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 225.

3 Shaklee, A. O., Christensen, K., and Kaplan, A., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 399.

4 Langley, J. N., *J. Physiol.*, 1905, **33**, 374; 1907, **36**, 347.

5 Dixon, W. E., and Ransom, F., *Ergebn. d. Physiol.*, 1912, **12**, 765.

TABLE I.
Data.

Heating, Temp.	0	35°-40°C	Additional Heating at 40°C						42°
'' , Durat. (min.)	0	15±	15	30	60	90	120	150	30
Cbmcl. (Const., %)	100	104	72	42	7	4	0.5	0	0
Atrop. (Dil., %)	100	102	64	35	8	7	1.7	0	0
Cooling (23°-0°) Const. %							5±	5±	0
Warming (0°-37°) Dil. %							10±	10±	0

Abbreviations: ''Durat.,'' duration; ''Cbmcl. (Const., %)'' gives the average amount of pupillary constriction to 3 drops of 0.3% carbaminoyl choline hydrochloride, expressed in % of the control; ''Atrop. (Dil. %),'' the subsequent average dilation to 2 drops of 0.01% atropine sulfate.

more than 6 successive litters, 42 fetuses, 84 eyes, 2136 careful measurements with a background consisting of a study of 99 other litters on the same general problem.

Findings and Tentative Conclusions. 1. Both the radial and constrictor muscle fibers, in the unheated eyes, and in those heated less than about 120 min at 40°C, possessed tonus; for after the pupil was constricted by carbaminoyl choline, paralysis of the cholinergic receptive substance by atropine, produced dilation. 2. The cholinergic receptive substance was completely destroyed by heating at 40°C, without destroying the contractile mechanism; for the amount of pupillary constriction that could be produced by carbaminoyl choline became progressively less as heating went on, until, at the end of about 120 min, it was entirely gone. Yet, after this complete destruction of receptive substance, cooling the eye produced constriction and warming produced dilation; while eyes in which the contractile mechanism had been "killed", by heating at 42°C for 30 min, gave no response to like changes in temperature.

Corollary: 1. The cholinergic receptive substance in the muscle fiber is independent of the contractile mechanism. 2. Carbaminoyl choline and atropine act on a cholinergic receptive substance in the sphincter of the iris. 3. These findings suggest further, that, in the process of "stimulating" a receptive substance in the living animal, the acetylcholine liberated from a nerve ending, may act as a catalyst on the receptive substance, causing it to discharge a second "stimulating" substance into the contractile mechanism of the muscle fiber. 4. The findings are in harmony with our former findings, and with the chemical theory of neuro-muscular transmission.

10550

Protective Antibody in Guinea Pigs Recovering from Experimental Pneumococcus Infection.

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In the course of observing various reactions associated with spontaneous survival of guinea pigs infected experimentally with pneumococcus Type I the appearance and rise of protective antibody in the blood has been studied. The animals were infected by intra-abdominal injection of 0.25 cc of an 18-hour serum-dextrose-broth culture of pneumococci. Under these conditions and without treatment 47 of 100 animals survived. The day of recovery of the animals has been considered as being identical with the day a negative culture of the peritoneal exudate was obtained. Only surviving animals were studied, since the majority which did not survive, died before the fourth day.

Blood was taken from the heart of the guinea pigs at various intervals and the serum separated. The tests were carried out by determining the number of minimal lethal doses of a virulent Type I pneumococcus which could be neutralized by 0.5 cc of the serum when mixtures of the culture and the serum were injected intraäbdominally into mice.

It was not possible to take blood from the guinea pigs for testing of protective titer at daily intervals so that there are of necessity some intervals which have not been thoroughly explored, but the studies are probably sufficiently detailed to warrant certain conclusions.

Forty-two of 47 guinea pigs which survived the infection are considered here. Serum for antibody-titration was obtained from all animals before infection. Only one animal showed the presence of antibodies; they were no longer demonstrable by the fifth day of infection and did not reappear during the period of observation (through the 24th day).

In Table I is shown the relation between the appearance of antibodies and the recovery from infection as measured by the negative peritoneal culture. In only a very few cases was blood taken for testing antibodies on the second day after infection, and in no animals were protective antibodies demonstrable. In 2 animals antibodies were demonstrable on the third day, in 5 animals on the fourth day, in 13 animals on the fifth day, and 2 on the sixth day, in 6 on the

TABLE I.
Day of Recovery and Day of Appearance of Protective Antibody.

Day of appearance of antibody / Day of negative culture	3	4	5	6	7	8	After 8	Negative	Total
1			2					1	3
2		3				1			4
3	1		1				1		3
4		2	5		4		2		13
5			4			3	1	1	9
6	1			1	1	1			4
7			1	1	1	1			4
8							1		1
9								1	1
Total	2	5	13	2	6	6	5	3	42

seventh day, in 6 on the eighth day, in 5 after the eighth day, and in 3 antibodies were not demonstrable at any time.

If one observes the general trend of the appearance of antibodies, it will be noted that few animals show antibodies before the fifth day, and that more animals have antibodies appearing on the fifth than on any other day; after the fifth day antibodies appear in considerable numbers of the animals. It may be assumed that the tendency is for the antibodies to appear regardless of the duration of the infection but that their appearance is affected by the continuance of the infection. This point is brought out if one considers the animals in which antibodies appear before or on the day of recovery; in the animals which recover early (before the fifth day) only 3 (13%) show antibodies by this time, while in the group recovering later (on or after the fifth day) 9 (50%) show antibodies before or on the day of recovery.

There is a possible relationship between the early appearance of antibodies (by or before the fifth day) and early recovery (by or before the fourth day). It need not be assumed that this relationship is between cause and effect, but it is quite possible that the early subsidence of the infection and diminution of the specific antigen in the invaded host permits the antibody to become evident.

In only a limited number of animals (3) is antibody demonstrable before recovery and then in the cases in which recovery was not before the sixth day. In 9 animals the antibody was demonstrable on the day of recovery. Thus in 12 animals (29%) antibodies were demonstrable before or coincident with recovery. In 21 of the 42 animals antibodies were demonstrable either before recovery, on the day of recovery, or on the first day after recovery.

In Table II is shown the range of the protective titer of the serum from the guinea pigs.

TABLE II
M.L.D.'s Neutralized.

	0	10	100	1000	10,000	100,000
3-5th day	20	13	5	1	—	1
6-9th "	6	5	5	3	14	5
10-19th "	6	2	2	5	7	6
20-33rd "	18	3	6	9	6	1

Relatively few animals show a high titer at any time, but there is evident the tendency towards reaching a maximal titer in the second or third week, followed by a fall in later periods. Many of the animals that had shown antibodies in the early periods do not possess these in their serum at later periods; at least 15 animals out of 33 which were studied over a sufficient period of time had lost their antibodies before the 33d day after infection and recovery.

In general these results concerning the appearance of antibodies in guinea pigs are in agreement with those observed in pneumococcal infection in human beings. It is not possible to predict the outcome of the infection through observation of the appearance of antibody or the level of antibody-titer.

10551

Mononuclear Leucocytes in Blood of Guinea Pigs Experimentally Infected with Pneumococcus.

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In view of the importance attached by many observers to the rôle of mononuclear cells in recovery from infection and especially the concepts advanced by Robertson and his coworkers[1-4] in pneumococcal infection in dogs, the changes in the mononuclear cells in the circulating blood have been studied in guinea pigs that died and that recovered spontaneously from experimental pneumococcal infection. The animals were infected by intraäbdominal injection of 0.25 cc of an 18-hour dextrose-serum-broth culture of Type I pneumococcus. Of 100 animals so infected 47 survived the infection. In this study

1 Robertson, O. H., and Uhley, C. G., *J. Clin. Invest.*, 1936, **15**, 115.
2 Robertson, O. H., and Loosli, C. G., *J. Exp. Med.*, 1938, **67**, 575.
3 Robertson, O. H., and Coggeshall, L. T., *J. Exp. Med.*, 1938, **67**, 597.
4 Robertson, O. H., *J. A. M. A.*, 1938, **111**, 1432.

TABLE I.

Mononuclear Counts in Animals Dying After the Second Day or Surviving the Experimental Pneumococcal Infection.

	0 hr	6 hr	1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day	9 day
Died bet. 2d and 3d day	350	300	288	121							
" " 3d and 4th "	350	225	342	148	645						
" " 4th and 5th day	266	400	118	457	608	1080					
" after 5th day	740	280	182	194	251	579	434	2608			
Group C	400	284	237	217	485	722					
Negative on 8th or 9th day	388	359	350	1387	905	651	464	987	701		1853
" " 7th day	342	234	440	169	1195	332	556		729		646
" " 6th "	451	371	258	481	1081	527	414	588		416	113
" " 5th "	392	361	131	369	311	828	480	309	414	611	1348
Group D	393	341	258	586	514	608	485	623	493	494	1181
Group E	308	261	293	229	591	588	409	311			727

of the mononuclear leucocytes 14 animals that died from 60 hours to 30 days after infection (Group C), 18 that recovered at 5 to 9 days after infection (Group D), and 14 that recovered on the fourth day (Group E) are considered. Recovery was assumed to have occurred on the day a negative culture was obtained from the peritoneal exudate.

At 6 hours after infection there was a decrease in the number of circulating mononuclears and this decrease was usually still evident or may have been more marked at 24 hours. The changes were observed in all groups regardless of whether death or survival follows (Table I).

In Group C, in those animals that died before the third but after the second day, the mononuclears continued to decrease after 24 hours; this change was concomitant with a decrease of total cells and of neutrophils.[5] Those animals that died on the fourth day showed a further decrease on the second day; however, on the third day these latter animals (which died within the next 24 hours) showed a distinct increase in the mononuclear cells. Other animals dying later than the fourth day, showed an increase at the second day and there was a tendency towards an increased number of cells in later days. In general if death was delayed beyond the third day, the number of mononuclears increased even though the animals died.

In Group D an increase in mononuclear cells occurred on the second day but was quite irregular as to magnitude in the different subgroups. From the second day onwards there was a tendency for the number of mononuclears to remain fairly high, but there was a considerable degree of variation in the different subgroups. There was no regularly observable rise in mononuclears in all of the smaller groups on the day before the negative culture; however, in the animals that became negative on the fifth day a rather sharp and distinct rise was observed on the fourth day; this is all the more striking since the mononuclears had remained at a fairly low level up to this day.

In Group E, in which all animals showed a negative culture on the fourth day, the count remained low on the second day and rose rather sharply on the third day, that is, just before recovery, remaining at about the same level on the date of the negative culture. Subsequently the mononuclear count fell.

Reviewing all 3 groups it appears that the mononuclears tend to follow a certain curve regardless of the outcome of the infection. Within the first 24 hours the counts are low, generally lower than

5 Fleisher, M. S., and Rich, G. T., to be reported.

before the start of the infection. On the second day the count rises, although the counts in the non-surviving animals tend to lag behind. By the third day the mononuclears in all animals or groups increase to a greater or less degree regardless of what the outcome will be, and this increase is usually maintained. In those animals that develop a negative culture on the fourth or fifth day the mononuclears usually remain low (at about the same level as before infection) up to the day before the negative culture, and on this date these cells show a definite increase.

It may then be assumed that changes in the number of mononuclear cells in the circulating blood during the earlier periods of the infection (during the first 24 hours) cannot be associated with and will not serve to predict the eventual outcome of the infection.

As regards the relationship of the increase of the mononuclears just before recovery the fact that a rise in these cells occurs at this same general period in most groups regardless of the outcome, raises doubt as to the interpretation of this reaction. It may be noted, however, that reaction of the mononuclears in animals recovering may be one of two types: (1) In the group that recovered on the fourth or fifth day there may be a definite association with an increase in the number of mononuclears as shown by the earlier low level and the sharp rise just before recovery; (2) in those animals that do not recover until after the fifth day, there does not appear to be any association between a rise of the mononuclears and immediate processes of recovery. If the mononuclear leucocytes are of importance in recovery from pneumococcal infection, it seems possible that their rôle is more definite in the first type of reaction, while in the second type of reaction they are less important.

10552

Serum Amino Nitrogen Concentration in Different Parts of the Vascular System.

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During investigations on amino nitrogen transport in the body a few studies were made on the amino nitrogen level in blood as it courses the vascular system. The observations were on dogs under

nembutal anesthesia after they had been fed Purina Dog Chow exclusively for one to 2 weeks and then fasted 24 hours. Venous blood samples, except those from the femoral vein, were obtained by passing a long straight cannula with a closely fitting stilet through an opening in the external jugular vein, down through the right auricle to the lower part of the inferior vena cava. Specimens were first taken from the lower and upper parts of the inferior vena cava (below and above the renal veins). With a little manipulation the cannula could then be placed and held snugly in a branch of the hepatic vein and venous blood from the liver thus obtained. Finally samples were removed from the right heart and superior vena cava. Three workers could obtain the 7 samples of blood, including the arterial, almost simultaneously. Blood was promptly centrifuged and amino nitrogen determinations made on the serum in duplicate by a modification of the Folin method.

The results are presented in Table I. In 4 (Nos. 1 to 4) the amino nitrogen in the venous blood from the liver was definitely lower than the arterial level. These have been averaged to contrast with 7 (Nos. 5 to 11) in which blood from the liver was approximately the same as the arterial. The averages are recorded graphically in Fig. 1. The magnitude of the changes is noteworthy.

There was, as a rule, a distinct rise in amino nitrogen concentration as blood passed from artery to the femoral vein and a further rise in the lower part of the inferior vena cava. Above the renal veins there was a decided fall. Renal amino nitrogen clearance studies have presented figures high enough to account for this drop. The concentration in the superior vena cava blood was only slightly

TABLE I.

Serum Amino Nitrogen in mg per 100 ml. Duplicate Determinations were as a Rule Identical and Only Those Figures Marked (?) Varied More than 0.2 mg.

	Femoral Artery	Femoral Vein	Lower, Inf. Vena Cava	Upper, Inf. Vena Cava	Hepatic Vein	Superior Vena Cava	Right Heart
1	5.1	5.7	6.7	6.7	4.8	5.6	5.1
2	5.9	6.1	6.3	5.7	5.2	5.9	5.9
3	5.2 (?)	6.1	5.6	4.8	4.4	5.4	5.2
4	5.4	5.5	6.4	5.5	5.0	5.6	5.4
Av.	5.4	5.8	6.2	5.7	4.8	5.6	5.4
5	4.3	4.8	4.8	4.5	4.2	4.1	4.3
6	4.6	5.9	6.0	4.7	4.7	4.7	4.4
7	5.7	5.7	6.7	5.7	5.8	5.8	5.6
8	4.8	5.3	5.5	4.9	5.1	5.0	4.9
9	4.9	5.2	5.5	5.1	5.0	5.0	4.9
10	4.6 (?)	5.1	4.8	5.1	4.9	4.9	4.9
11	5.1	5.7	5.8	5.1	5.1	5.5	5.1
Av.	4.9	5.4	5.6	5.0	5.0	5.0	4.9

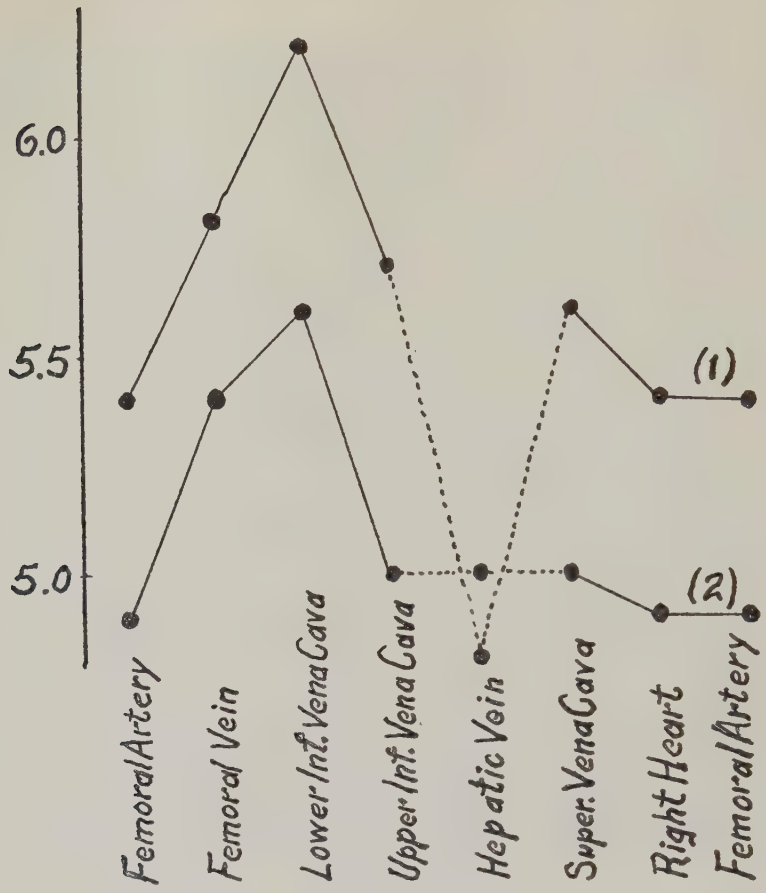

FIG. 1.

Showing relative concentrations of serum amino nitrogen of arterial blood and of blood from various parts of the venous system. In group (1) the concentration in blood from the liver was lower than the arterial level. In group (2) they were at the same level.

greater than in the arterial. That of the right and left (arterial blood) side of the heart was usually the same, thus indicating no change in amino nitrogen level in the pulmonary circuit.

Comparing (Fig. 1) those experiments in group (1) in which the amino nitrogen concentration in the blood from the liver was distinctly lower than the arterial level (suggesting rapid removal by the liver) with those of group (2) one notes no striking difference except that the average level of amino nitrogen was definitely higher in the former.

10553 P

A Study of Hereditary Chondrodystrophia in the Chick ("Creeper" Fowl) by Means of Embryonic Transplantation.

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Landauer and Dunn[1] have shown that the "Creeper" variety of fowl (characterized by short legs) is the manifestation of a single dominant Mendelian factor in heterozygous condition (Cpcp). The factor in homozygous condition (CpCp) is usually lethal on the 4th day of incubation; however a few CpCp embryos survive and exhibit a complex of symptoms: phocomelus, coloboma, microphthalmia, etc.[2] Laudauer suggested that the primary cause underlying these malformations is a general growth inhibition and that the morphogenetic and histogenetic disturbances are secondary effects.

When normal leg primordia of 2-3 day embryos are transplanted to the flank or into the coelom of normal embryos of the same stages,[3] a growth inhibition of the transplants results which, on the average, is similar in magnitude to the one found in Cpcp-embryos of identical stages. Although skeletal defects are frequent in these transplants, we have never found the type of malformation characteristic for Creeper legs. Obviously, a general growth inhibition does not result necessarily in Creeper-like deformities. Either the inhibitor postulated by Landauer acts prior to the stage of transplantation, or we are dealing with 2 growth-restricting agents which affect different components of the growth process.

In the present experiments, an attempt was made to determine whether the "Creeper" factor acts locally in the limb forming areas or is indirect in its action. Leg primordia of Cpcp-embryos and wing and leg primordia of CpCp-embryos (48 to 72 hrs of incubation) were transplanted to the flank or into the coelom of normal embryos (White Leghorn) of the same stages.

1. The best developed cases of the 28 *Cpcp*-transplants, raised 10 to 17 days, showed all typical Creeper characteristics; bending of the tibia, abnormally long fibula, etc. In many cases, the malformations observed in these transplants were more marked than in Cpcp-embryos: excessive growth reduction, extreme shortening and fusion

1 Landauer, W., and Dunn, L. C., *J. Genetics*, 1930, **23**, 397.

2 Landauer, W., *J. Genetics*, 1932, **25**, 367; *Z. f. Mikr.-Anat. Forsch.*, 1931, **25**, 115; 1933, **32**, 359.

3 Hamburger, V., *J. Exp. Zool.*, 1938, **77**, 379; 1939, **80**, 347.

of tibia and fibula. Other elements (femur, tarsals, phalanges) were less seriously affected. Such transplants resembled the "phocomelus" condition found in surviving CpCp-embryos. Hypodactyly was frequent. Ossification was normal. Since normal (cpcp) and Cpcp-embryos cannot be distinguished at the time of operation, the genetic constitution of the transplants (as Cpcp) was verified in 7 cases by raising the donor to advanced stages. Structurally normal transplants (from cpcp donors) were found in 8 out of 36 cases. The genetic constitution (as cpcp) was verified in 2 of them by raising the donor. A proportion of 24 Cpcp to 12 cpcp transplants would be expected. The ratio found in these experiments (28:8) is thus not a serious deviation from the expectation.

2. Wing and leg transplants from *CpCp*-embryos (recognizable at the stage of operation) survive in the normal host beyond the lethal stage of the donor. This potentiality was shown previously by the occurrence of surviving CpCp-embryos[2] and in tissue culture experiments.[4] All 29 transplants, (CpCp) raised from 10 to 17 days were smaller and more abnormal than Cpcp-transplants. The best developed transplants resembled the "phocomelus"-type with short proximal parts and relatively well developed toes. Hypodactyly was common. In most cases, all skeletal elements were bent or distorted and fused together, forming one complex in which the individual parts were more or less clearly distinguishable. Other transplants consisted of atypical outgrowths. They contained cartilages of atypical shape. Enchondral ossification was absent even in transplants which were found in healthy condition on a highly ossified 17-day-old host.

Conclusions. 1. The "Creeper"-factor, both in homozygous and in heterozygous condition, acts locally in the limb-forming areas at least from the stage of the first visible appearance of the limb buds on. 2. In particular, the possibility that the Cp-factor acts indirectly by causing a deficiency of any substance contained in the blood circulation (nutritive, hormones, Ca, etc.) is ruled out. The transplantations were made shortly after onset of circulation. 3. Wing and leg primordia of early lethal CpCp-embryos differentiate into "phocomelic" appendages if transplanted to a normal host. This confirms Landauer's[2] findings that "phocomelus" is the manifestation, in the appendages, of the Cp-factor in homozygous condition. 4. The fact that CpCp-limb primordia survive the critical stage of lethality if incorporated in a normal host suggests that the cause of lethality resides not in the limb-forming area but in another structure outside of it.

[4] David, P. R., *Roux' Arch. f. Entw.*, 1936, **135**, 521.

10554 P

Relative Efficiency of Commercial Forms of Insulin.

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Protamin zinc insulin preparations are known to exert an effect more prolonged than that of equal unitage of unmodified (regular) insulin. Recently claims have been offered that the effect of insulin in the form of zinc insulin crystals is similarly prolonged, but in lesser degree. The issue has been confused through the use of clinical observations as the basis for reported conclusions. In view of the many factors which can influence the quantitative response to insulin, the need for standardized conditions of study is apparent. If human subjects are to be used, the constancy of insulin requirement should be established through the use of prolonged control periods wherein daily fluctuations of blood sugar are known.

It has been demonstrated recently[1] that with suitable treatment, the insulin requirement of the diabetic child decreases sharply, once aglycosuria is established, the final daily insulin requirement being 30 to 70% lower than that commonly given under customary methods of treatment. Once such stabilization has been accomplished, the child's insulin requirement is relatively constant and he can be maintained for long periods of time in a state of aglycosuria, with relative freedom from shocks and with blood sugar values which approach those of the normal non-diabetic child. Such a subject is eminently suitable for the study of variations in effectiveness of different insulin preparations. The amount of fluctuation of blood sugar values during the 24 hours can be used as an objective method of comparison.

The diurnal blood sugar fluctuation of 12 diabetic children has been studied when the insulin requirement has been given as regular insulin, protamine zinc insulin, and zinc insulin crystals. The studies have involved a total of 1074 days and 1967 determinations of blood sugar.

It was not possible to stabilize the insulin requirement of diabetic children through the use of protamine zinc insulin. Once stabilized, however, when the total insulin requirement did not exceed 1 unit

[1] Jackson, R. L., and Boyd, J. D., Trans. Soc. Clin. Research, Chicago, Nov., 1938, *J. A. M. A.*, 1939, **112**, 1017.

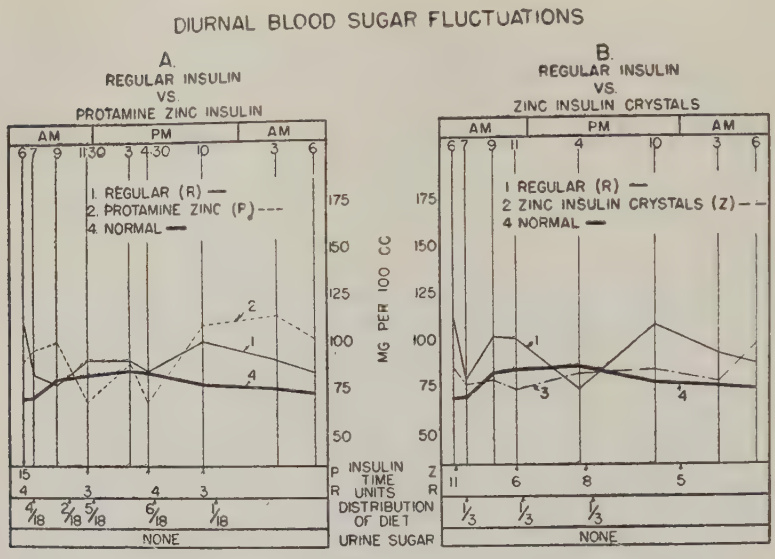

FIG. 1.

Illustrative diurnal blood sugar fluctuation in stabilized diabetic children receiving equivalent unitage of various types of insulin, other factors constant. In each instance the regimen had been constant for several days before the blood sugar values were determined.

A. Blood sugar fluctuations of 10-year-old diabetic girl given
   (1) regular insulin
   (2) protamine zinc insulin

B. Blood sugar fluctuations of 13-year-old diabetic girl given
   (1) regular insulin
   (2) zinc insulin crystals

The heavy line 4 in each chart indicates average blood sugar values observed in non-diabetic children under a comparable dietary regimen. The Folin-Wu blood sugar method was employed, using blood from the finger and Somogyi's method for precipitation of non-sugar reducing substance.

for each kilogram of body weight or a total of 20 units daily, single doses of protamine zinc insulin maintained the blood sugar at approximately normal levels as is shown in Chart A.

When zinc insulin crystals* were substituted for regular insulin, in the same unitage and using the same time of administration (Chart B), no clinically significant prolongation of action was observed. It was possible to interchange regular insulin and zinc insulin crystals at will and repeatedly without altering the customary zone of diurnal blood sugar fluctuation.

* Supplied through the courtesy of Eli Lilly and Company.

10555

Effects of Sulfanilamide, Phenothiazine and Thionol in Experimental Trichinosis.*

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It is almost impossible to evaluate the efficacy of the various drugs recommended for the treatment of human trichinosis. Animal experimentation appears to be the only feasible solution to the problem of studying the effects of therapeutic agents in this disease. This paper deals with the effects of sulfanilamide, phenothiazine and thionol on experimental trichinosis in rats.

Methods. Sixteen adult white rats of the Wistar strain averaging 190 g in weight were segregated into 4 groups of 4 each. After one week of observations on the quantity of standard dry rat diet consumed by each group they were placed on diets to which known quantities of the drugs being studied had been added. They were allowed to eat this diet for 2 days in order to develop some concentration of the therapeutic agents prior to infection. After an 18-hour fast each rat was infected by a quantity of rat meat containing 2,350 encysted larvae. The number of living larvae per gram of rat meat was calculated by multiple counts on multiple digests of thoroughly minced muscle from rats according to methods previously described.[1] The strain of *Trichinella spiralis* had been originally obtained from a human diaphragm. The rats were kept in cages in which the feed cups were designed to prevent losses, so as to permit determination of the amount of food consumed each day. Water was available at all times. One group of rats on standard diet served as controls; the other 3 groups were kept on the same diet plus the drugs being studied. The various agents in powder form were thoroughly mixed with the feed in a mechanical mixer. Rat weights and food consumption were recorded twice weekly for 6 weeks. All rats were then sacrificed, their carcasses individually digested and an estimate made of the total number of larvae which had developed in the muscles of each.

*Supported in part by the Rockefeller Fluid Research Fund of the School of Medicine of Stanford University.

1 McNaught, J. B., and Pierce, G. N., Jr., *Am. J. Clin. Path.*, 1939, **9**, 52.

TABLE I.
Effects of Sulfanilamide, Phenothiazine, and Thionol on Trichinosis in Rats.

Drugs	Group* wt Initial, g	Group* wt Final, g	Total food Consumed, g	Avg daily drug intake per kilo body wt, g	No. of Larvæ recovered	Reduction in infestation, %
Controls	797	955	1,931		762,400	
Sulfanilamide	986	985	2,263	.96	343,120	55
Phenothiazine	633	746	1,512	.14	198,720	74
Thionol	751	783	1,680	.13	421,760	45

*Four rats in each group. Each rat was infected by 2350 encysted larvæ.

Controls. Four male rats were used as controls. Table I shows the pertinent data for this group which gave an intake-output ratio of 1 to 81.1 larvae. Each gram of skinned, eviscerated rat contained 1,463 larvae.

Sulfanilamide. Inasmuch as sulfanilamide has been shown to be a beneficial therapeutic agent in certain bacterial infections and since its value in parasitic diseases can be determined only by trial it seemed worthwhile to test its effectiveness in trichinosis. Powdered sulfanilamide (p-aminobenzenesulphonamide—Prontylin—Winthrop) was added to the standard diet to a concentration of 1.66%. Male rats were used in this experiment. Table I shows only 45% as many larvae recovered as in the control group. The intake-output ratio was 1 to 36.5. There were 639 larvae per gram of dressed rat.

McCoy[2] in a similar experiment on rats, infected by excysted Trichinella and receiving a dose of 125 mg of sulfanilamide daily for 16 days beginning the day after infection, found no material difference in the final larvae counts between the treated and control animals. In a second experiment his figures do show a decrease of 30% in the total count of larvae in the treated group. His conclusions were that sulfanilamide had no value in treatment of experimental trichinosis in rats. Our figures showing a decrease of 55% are possibly accounted for by the fact that we gave larger doses, continued therapy for 42 days instead of 16 days and began medication two days prior to infestation.

Phenothiazine. Phenothiazine has shown promise as an insecticide[3] and as a urinary antiseptic.[4] It has been reported to give 100% control of larvae of the horn fly, *Haematobia irritans L.*, developing in the manure when administered to cattle at the rate of 0.1 g or less

2 McCoy, O. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 461.

3 Smith, L. E., Munger, F., and Siegler, E. H., *J. Econ. Entomol.*, 1935, **28**, 727.

4 Thomas, J. O., DeEds, F., and Eddy, C. W., *J. Pharm. Exp. Therap.*, 1938, **64**, 280.

per kilo of body weight.[5] In view of these findings it seemed advisable to try the effects of this drug on trichinella in the gastrointestinal tract. Accordingly parasitized female rats were fed the standard dry rat diet to which .25% phenothiazine was added.

Table I shows that this group yielded only 26% as many larvae as did the control group, thus indicating that phenothiazine in the quantities and manner given furnished 74% protection from experimental trichinosis in rats. There apparently were no ill effects from the continued use of this drug. The total dose of phenothiazine was only a tenth of the total dose of sulfanilamide.

There was a final yield of 493 larvae per gram of dressed rat and an intake-output ratio of 1 to 21.1.

Following completion of this study our attention was called to the report of Harwood, Jerstad, and Swanson[6] on the beneficial results following the use of phenothiazine for the removal of ascarids and nodular worms from swine.

Thionol. It has previously been shown[4] that the bactericidal properties of urine following oral administration of phenothiazine are to be attributed to thionol, an oxidation product of phenothiazine. It therefore seemed advisable to compare the efficacy of this compound with the parent substance, phenothiazine. The thionol used was prepared from phenothiazine by a simple process of oxidation recently described.[7]

Four parasitized female rats were fed the standard dry rat diet containing .25% thionol. Table I shows that this group yielded 55% as many larvae as did the control group, thus indicating that thionol in the quantities given furnished 45% protection from trichinosis and was far less efficacious than phenothiazine. There was a final yield of 974 larvae per gram of dressed rat and an intake-output ratio of 1 to 44.8.

An interesting observation in these experiments concerns the maintenance of weights of the animals and their food consumption. All groups lost weight the first 2 weeks of infestation because of their diminished food intake during the acute stage of the disease. All except those on sulfanilamide had returned to their original weights by the end of the third week and some showed up to 20% gain by the close of the experiment. More grams of food were consumed per gram of rat in the group on sulfanilamide than in any other group

5 Knipling, E. F., *J. Econ. Entomol.*, 1938, **31**, 315.

6 Harwood, P. D., Jerstad, A. C., and Swanson, L. E., *J. Parasitol.*, Supplement, 1938, **24**, 16.

7 DeEds, F., and Eddy, C. W., *J. Am. Chem. Soc.*, 1938, **60**, 1446.

yet these rats just succeeded in attaining their original weights by the final week. This suggests that some factor in sulfanilamide did not permit the normal utilization of the diet in trichinous rats. McCoy noted that infected rats lost considerably more weight under sulfanilamide therapy than control rats and also more than other rats which were given the same amount of drug but were not infected with Trichinella. There is a possibility in our experiment that there was some loss of food among the shavings of the rat cage in the case of sulfanilamide due to dislike for the food. It is interesting that although the control rats carried a heavier parasitic infestation than any other group they made the greatest gain (20%) over their original weight. The rats on phenothiazine made a gain of 18%.

Conclusions. (1) A rather large amount of sulfanilamide, 0.96 g per kilo of body weight daily, used over a period of 6 weeks reduced the number of trichinella encysting in the muscles of rats by 55%. (2) The continuous use of phenothiazine, in a dosage approximately one-tenth that of sulfanilamide, over a period of 6 weeks reduced the severity of trichinous infection in rats by 74% and warrants further experimentation. (3) Thionol is of little use in reducing the severity of trichinous infestation in rats.

10556

Phosphorus Metabolism in Leukemic Blood.

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The fact that radioactive phosphorus is chemically like ordinary phosphorus and is "tagged," has made it valuable in following the exchange of phosphorus in biological systems.[1] The present study is concerned with the absorption, distribution, and excretion of labelled phosphorus (P^{32}) in 2 patients suffering from chronic myelogenous leukemia. The mixture of P^{31} (inactive) and P^{32} (radioactive) atoms were converted into Na_2HPO_4 and administered orally in an isotonic solution of this salt.

The first patient studied was a case of untreated chronic myelogenous leukemia, in fair clinical condition. The white blood count

1 Lawrence, J. H., Artificial Radioactivity and Neutron Rays in Biology and Medicine, *Handbook of Physical Therapy*, Am. Med. Assn., 1938.

was 195,000 cells per mm^3, and the red cells numbered 3.7 million. The differential count was as follows: staff 40%, segmented 26%, metamyelocytes 9%, neutrophilic myelocytes 12%, eosinophilic myelocytes 1.5%, eosinophiles 1.5%, basophiles 0.5%, progranulocytes A 0.5%, S 0.5%, blasts 2.5%, lymphocytes 3.5%, mononuclears 2.5%. Under fasting conditions a tracer dose of 2.98 millicuries of radiophosphorus was given by mouth in an isotonic solution containing 0.8 g of Na_2HPO_4. Urinary and fecal excretion of radiophosphorus was determined daily for a period of 9 days. During the first 3 days 13.4% of the total activity administered was excreted in the feces and 8.1% in the urine. During the next 6 days, the daily urinary excretion was 0.90%, .99%, .84%, .80%, .83%, and .76% of the tagged phosphorus, while the daily fecal excretion was .30%, .17%, .22%, .21%, .10%, and .12%. At the end of 9 days 72.2% of the total dose was retained.*

The second patient, who was suffering from myelogenous leukemia of 2½ years' duration, and who had not received X-ray or other treatment for 4 months, was also in fair clinical condition. The white blood count was 163,000, and the red cells numbered 4.4 million. The differential count was as follows: staff 35%, segmented 16%, metamyelocytes 6.5%, neutrophilic myelocytes 12%, eosinophilic myelocytes 9.0%, staff eosinophiles 5.0%, basophiles 1.5%, eosinophiles 2.0%, blasts 2.5%, progranulocytes A 3.0%, lymphocytes 2.0%, mononuclears 3.0%, and unclassified 2.5%. Under fasting conditions a dose of 4.7 millicuries of radiophosphorus was given by mouth in an isotonic solution of 3.0 g Na_2HPO_4. During the first 3 days, 25.6% of the dose was excreted in the feces and 16.5% in the urine. During the next 9 days a total of 7.8% was excreted in the urine, and 1.48% in the feces. Of the dose given, 48% was retained at the end of 12 days.

The technic of separation of blood into its red and white cell fractions was as follows: In each case, 15 cc of blood were drawn, heparinized with 1 mg heparin† to 15 cc whole blood, and immediately centrifuged for exactly 20 minutes at a force of 1450 times gravity. Under this treatment, the blood separated into 3 distinct layers: plasma, white cells, and red cells. The plasma was removed and set aside for analysis. Next, a portion of the white cell layer was removed and suspended in isotonic Ringer's solution. Similarly, a portion of the red blood cell layer was suspended in Ringer's solution. Then both cell suspensions were again centri-

* All activities were corrected for decay.

† Connaught Laboratories, University of Toronto, Canada.

TABLE I.
Distribution of Radioactive Phosphorus in Leukemic Blood.

Case I	White Blood Cells			Red Blood Cells			Whole Blood			Plasma		
Days After Admin.	% dose per 100 cc	Mg P per 100 cc	Microcuries* per 100 mg P	% dose per 100 cc	Mg P per 100 cc	Microcuries per 100 mg P	% dose per 100 cc	Mg P per 100 cc	Microcuries* per 100 mg P	% dose per 100 cc	Mg P per 100 cc	Microcuries per 100 mg P
2	.230	234	2.94	.148	80.5	5.50	—	—	—	.021	12.2	5.22
4	.214	226	2.82	.105	83.0	3.78	.061	54.0	3.39	.015	12.6	3.42
9	.212	206	3.04	.053	84.0	1.90	.047	53.8	2.61	.012	12.5	2.96
14	.253	204	2.69	.053	85.0	1.87	.044	54.7	2.36	.0094	12.4	2.25
53	.136	227	1.79	.029	70.7	1.24	.022	52.0	1.26	.0075	15.0	1.51
Case II												
½	.20	261	3.58	.220	64.5	15.8	.125	64.5	9.11	.023	16.8	6.30
2	.23	262	4.17	.110	75.0	6.94	.078	—	—	.021	20.0	4.90
4	.29	—	—	.065	72.5	4.18	.060	62.2	4.52	.015	17.3	4.21
9	.30	257	5.53	.049	72.5	3.21	.059	67.6	4.08	.0115	16.9	3.22
14	.24	—	—	.042	70.0	2.80	.043	48.5	4.17	.0088	16.2	2.58

*All activities corrected for decay.

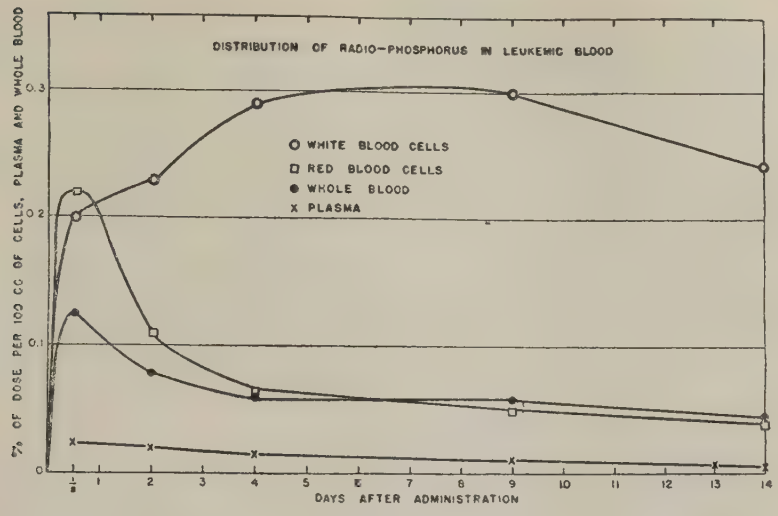

FIG. 1.

fuged for 20 minutes at 1450 times gravity under exactly the same conditions as before. The supernatant wash solution was removed from each cell fraction and set aside for an analysis. The washed red and white cells were then carefully aspirated into graduated capillary pipettes, and their volumes measured. After the samples were ashed at 450°C, the radioactivity of each was measured by means of a Lauritsen electroscope. Total phosphorus was determined by titration after precipitation as ammonium phosphomolybdate according to the method of Pregl.[2]

It should be emphasized that the values presented here do not pretend to be absolute, but do demonstrate the relative phosphorus metabolism of red and white cells that have been handled in an identical and reproducible manner. The technic was checked by means of blood counts on smears made from the final washed preparations. In the second case studied, counts made on the five successive white cell fractions indicated that white blood cells made up 59, 62, 57, 57, and 58%, respectively, of the total number of cells. Considering the relative sizes of red and white cells, this indicates that roughly 90% of the volume of the white cell fractions was composed of white cells. Similarly, it was established that the washed red cell fractions were contaminated with less than 1% white cells.

The amount of radioactivity found in the supernatant wash solution from each cell fraction in no case exceeded 10% of that found

2 Pregl, F., and Roth, H., *Quantitative Organische Mikroanalyse*, Julius Springer, Berlin, 1935.

in the cells themselves. The range for white cell washings was from 5.2% to 10%, averaging 7.0%, and for red cell washings 3.8% to 10.0%, averaging 7.3%. Thus it is seen that the cells lost no significant amount of their phosphorus as a result of washing.

The data are presented in Table I. In Fig. 1,‡ the amount of radioactive phosphorus found in equal volumes of cells, plasma and whole blood is expressed as percent of total dose and plotted against time in days.

During the hours soon after administration, red cells exchange phosphorus much more rapidly than do the white cells, indicating that the phosphorus is more concerned with function than with structure. In the case of the white cells, there is a rapid initial uptake, subsequent to which the activity curve rises slowly over a period of days, and then gradually falls off.

The high rate of metabolic turnover of phosphorus in red blood cells, best shown by Fig. 1, may be attributed: first, to the anion shift in neutrality regulation in which inorganic phosphate plays a rôle; second, to their function as a temporary storage vehicle for phosphate, as suggested by Buckman, *et al.*,[3] and third, to their function in glucose utilization in which phosphate is concerned, as shown by Halpern.[4]

The rapid initial uptake of phosphorus by the white cells is possibly due to their glycolytic function. The continued slower rise and retention of phosphorus over a long period of time is probably conditioned by the formation of new cells in which the phosphorus may be held in nucleoprotein in a relatively stable state.

The diet of the 2 patients, both of whom were able to pursue their normal occupations, was not controlled as to total phosphorus and calcium intake; neither were their total phosphorus and calcium excretions determined. The data are concerned only with the absorption, distribution and excretion of a single dose of "tagged" phosphorus taken by mouth under fasting conditions.

Three days after administration, the first patient had retained approximately 80% of the radioactive phosphorus given. The daily excretion thereafter averaged about 0.9% in the urine and 0.2% in the feces, a ratio of 4 to 1. The second patient, whose clinical condition was somewhat the better of the 2, had retained only 60% of the dose at the end of 3 days. The subsequent daily excretion was

‡ These curves are obtained from the data of Case 2. Case 1 gives similar curves.

3 Buckman, T. E., Daland, G. A., and Weld, M., *Arch. Int. Med.*, 1925, **35**, 389.

4 Halpern, L., *J. Biol. Chem.*, 1936, **114**, 747.

less than 1%; the urine-to-feces ratio being about 5 to 1. The lower absorption by the second patient may be ascribed to the fact that he was given a larger total dose (3 g) of Na_2HPO_4, which may have acted as a mild cathartic and hastened the passage of the ingested material through the small intestine.

It is to be noted that in the first patient 26% of the cells were young forms including metamyelocytes, myelocytes, and a few blasts. In the second patient, 33% of the cells were of such forms. Thus we are unable to state that the handling of phosphorus observed in these cases is characteristic of normal white cells, but the red cell findings may be assumed those of normal red cells.§

10557

Use of Liver Extract in Place of Yeast in Low Fat Diets.*

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During our studies on the B vitamins in liver extract, we found that definite retardation of growth and development of specific symptoms resulted when our synthetic diet was supplemented with liver extract without the addition of fat. The relation of fat to the normal development of the rat has been approached by several methods. Fats have been found to cure the dermatitis produced in rats on highly synthetic diets free of the B complex but containing B_1 and flavin.[1, 2] However, on such diets very little growth is obtained when the fat is added. With rations containing ether-extracted yeast to supply the B complex, symptoms of fat deficiency, namely scaly tail and paws, appear in 4-8 months. Upon addition of fat at

§ The radiophosphorus used in these studies was produced by the cyclotron, through the generous cooperation of the staff of the Radiation Laboratory. We acknowledge with thanks also grants-in-aid from the Josiah Macy, Jr., Foundation. Assistance from the W.P.A. is also acknowledged.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Research Funds of the University. We are indebted to the W.P.A. project No. 8649, for assistance in care of animals.

1 Quackenbush, F. W., Platz, B. R., and Steenbock, H., *J. Nutrition*, 1939, **17**, 115.

2 Birch, T. W., *J. Biol. Chem.*, 1938, **124**, 775.

this stage a small growth response and alleviation of the deficiency symptoms result.[3–5] Both types of deficiency have been cured by purified preparations of linoleic acid. Since there is still a great interest in the nutritional significance of this and other fatty materials, we felt that our experience with liver extract might be of some value to workers interested in the fat problem.

The basal diet, designated J_{35}, used in this work consisted of sucrose 78%, casein 18%, and salts 3† 4%. In addition, each rat received daily supplements of 10 gamma of thiamin, 20 gamma of riboflavin, 1 mg of choline and 500 mg of liver extract powder. Twelve mg of haliver oil were given each week.‡ The casein was purified by washing and reprecipitation followed by 4 hot alcoholic extractions. The liver extract powder was the water soluble fraction (1 part equals 20 parts whole liver) prepared by Wilson and Company.§

Rats from our stock colony were used in all of these experiments. The young rats with their mothers were placed on raised screens during the last week of the suckling period. The young were given access to the experimental diet only and the mothers were removed from the cage each day for feeding. This enabled us to produce rats with a uniformly low storage of fat. This was necessary, for otherwise some litters continued to grow and failed to show the deficiency symptoms to be described below.

On this ration growth is fairly good for the first few weeks. The animals show a growth plateau at weights ranging between 80 and 140 g. Since our ration contained liver extract instead of the usual yeast, we will describe the appearance and condition of the rats in detail.

The deficiency was manifested roughly in both acute and chronic forms. In the acute form growth ceased very abruptly. A scaliness and reddening of the paws then occurred. The animals became very weak and emaciated and showed a marked loss of muscle tonus. There was occasional diarrhea. An extremely rapid loss in weight

[3] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

[4] Martin, G. J., *J. Nutrition*, 1939, **17**, 127.

[5] Hume, E. M., Nunn, L. C. A., Smedley-MacLean, I., and Smith, H. H., *Biochem. J.*, 1938, **32**, 2162.

† The salts 3 are the same as salts 1[10] with an additional 1.21 g $MnSO_4 \cdot 4H_2O$ per kilo of salt mixture.

[10] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

‡ The haliver oil was kindly supplied by Dr. C. Nielsen, Abbott Laboratories, North Chicago.

§ The liver extract was generously furnished by Dr. David Klein, Wilson and Company, Chicago.

TABLE I.
Growth Response to Supplements of Various Fats.

Rat No.	Sex	Supplement	Wt when test started	Gain in in wt	Avg daily gain	Duration of test (days)	Type of deficiency
13712	♀	0	103	— 2	—0.2	11*	acute
13716	♂	2 drops corn oil per day	62	98	2.8	35	,,
13814	♂	0	112	—	—	—*	,,
13845	♀	3 drops coconut oil per day	122	11	0.3	35	chronic
13846	♂	0	90	— 31	—4.8	7	acute
13382	♀	3 drops coconut oil per day	110	— 9	—1.8	5	,,
14261	♀	350 gamma of tocopherol per wk.	125	7	0.4	19	chronic
14383	♀	3 dr. butterfat per day	118	— 5	—0.2	20	acute
13726	♀	4 ,, linseed oil ,, ,,	83	75	2.1	35	chronic
13725	♂	2 ,, ,, ,, ,, ,,	72	112	3.2	35	,,
13721	♀	0	104	—	—	—*	acute
12951	♂	3 dr. corn oil per day	129	63	3.0	21	,,
12952	♂	3 ,, ,, ,, ,, ,,	129	59	2.8	21	,,
12954	♂	3 ,, butterfat ,, ,,	121	35	1.7	21	,,
12956	♀	3 ,, ,, ,, ,,	86	20	1.0	21	,,
11841	♀	3 ,, wheat germ oil per day	118	60	1.8	21	,,
11843	♂	3 ,, ,, ,, ,, ,, ,,	122	79	3.8	21	,,
11844	♂	3 ,, "snowdrift" per day	137	65	3.1	21	,,
11845	♀	3 ,, ,, ,, ,,	132	53	2.5	21	,,

*Animals died.

then ensued and nasal hemorrhages and cyanosis preceded death. A quick recovery would follow if certain fats were given before the animals became too weak.

In the chronic form the plateau in the rate of growth was delayed or incomplete. A severe scaliness of the tail and paws, typical of that described by Burr and Burr[3] occurred. Also a progressive atrophy of the testes in the male and a delayed opening of the vagina in the female was a common occurrence. A severe eczema of the back and neck and an erosion and erythema about the eyes was seen less frequently. These animals did not show the rapid weight loss and sudden death that was shown in the acute form.

Rats in the above condition could be rapidly restored to normal by the addition of 2 or 3 drops daily of corn oil, linseed oil, or wheat germ oil. The growth response in most cases averaged at least 3 g per day and the deficiency symptoms rapidly disappeared. Muscle tonus and general activity was quickly restored. Although the animals grew rapidly, the scaly tail condition was very slow to heal. Table I gives examples of the responses obtained. Butterfat gave intermediate responses while coconut oil and α-tocopherol‖ gave little or no response.

Necropsies of the animals that died of the acute deficiency showed normal lungs, livers, and kidneys. There was impairment of the reproductive organs. The testes, prostate and seminal vesicles were markedly atrophied. Hatai[6] has found atrophy of the testes on low fat diets.

To obtain maximum responses to concentrates of the vitamin B complex, fat must be present in the diet. To demonstrate this, certain concentrates were fed to rats receiving ration J_{30}, described by Oleson, *et al.*,[7] with and without fat. A crude concentrate of factor W (alcohol ether precipitate) was prepared by the method of Elvehjem, *et al.*,[8] and fed with and without the addition of 2 drops of corn oil per day to each rat. Factor 1 (vitamin B_6) and factor 2 (filtrate factor) concentrates[9] were fed on the same diet with and without the inclusion of 10% lard. These concentrates were kindly supplied to us by Dr. Lepkovsky. Table II shows the increased growth responses obtained from these concentrates when fat was present in the diet as lard or corn oil.

‖ We are indebted to Dr. R. T. Major, Merck and Company, Rahway, New Jersey, for a sample of α-tocopherol.

6 Hatai, S., *Anat. Res.*, 1915, **15**, 1.

7 Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

8 Elvehjem, C. A., Koehn, C. J., and Oleson, J. J., *J. Biol. Chem.*, 1936, **115**, 707.

9 Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

TABLE II.

Group	Ration and Supplements	No. of rats	Avg initial wt	Avg wt at 6 weeks
1	Ration J_{30} + ¼ g alcohol-ether ppt./day (factor W)	3	38	96
2	Same as (1) + 2 drops corn oil/day	3	40	153
3	J_{30} + 0.5 cc factor 2 + .05 cc factor 1 (B_6) concentrates	3	42	109
4	Same as (3) + 10% lard	3	44	189

Summary. When our results are compared with those of other workers quoted above, it is evident that the use of liver extract instead of the usual yeast in studies on low fat diets results in an earlier and more acute deficiency and hence a greater growth response when fat is given. Possibly the liver extract is lower than ether-extracted yeast in the essential fatty acids. This fact may be useful in working out better methods for the assay of at least some of the essential fatty acids in fats. Furthermore, it is necessary to have fat in the diet to obtain maximum growth responses to the liver extract concentrates of the vitamin B complex.

10558 P

Attempted Quantitative Estimation of Atabrine Retardation of Schizogony in Avian Malaria.

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Employing female canaries, mosquito infected with *Plasmodium cathemerium* (Hartman) and kept in the dark from 6 P.M. to 6 A.M. and in the light during the other 12 hours, I have found the following features to characterize the peripheral blood picture at 1 P.M.: (a) the majority of the schizonts in singly infected cells are not yet fully segmented though well advanced in size, and they are contained predominantly in mature erythrocytes; (b) pigmentation is pronounced; (c) vacuolation of an apparently degenerative nature is seen occasionally in the larger but not in the smaller forms.

Atabrine,* given repeatedly in adequate therapeutic dosage, alters this picture as follows: The total number of schizonts is reduced;

* Atabrine for this study was kindly supplied by the Winthrop Chemical Company, Inc.

TABLE I.
Distribution of Schizonts in Adult Erythrocytes Before Atabrine.
Bird 89, 1-14-39.

Size (increasing from A to D)	No. in singly infected cells	%	% large (C + D)	% small (A + B)
A	7	3.5		3.5
B	29	14.5		14.5
C	53	26.5	26.5	
D	111	55.5	55.5	
Totals	200	100.0	82.0	18.0

the amount of pigment is diminished; vacuolation appears in the younger as well as in the older forms; and the proportion of large to small schizonts in mature erythrocytes is reversed.

Reducing the amount of atabrine to a single dose given at midnight, I have found that when this single dose is as small as 0.1 mg per 20 gm of bird the total number of schizonts in single infected cells at 1 P.M. next day is not reduced, but pigment diminution, vacuolation, and the reversed preponderance in size of schizonts in mature erythrocytes do occur. Since vacuolation and pigment diminution are qualitative but not quantitative evidences of the drug's action, I am tentatively looking upon the amount of reversal from large to small schizonts in mature singly infected erythrocytes, occurring without reduction in the total number of schizonts in singly infected cells, as potentially a quantitative measure of the drug's ability to retard the process of schizogony. Borrowing the term of Moshkovsky and Poliakova,[1] such a dose might be called the *dosis affectans*.

Table I shows the typical distribution of schizonts in singly infected adult erythrocytes at 1 P.M. and Table II the altered distribu-

TABLE II.
Distribution of Schizonts in Adult Erythrocytes After Atabrine (*Dosis affectans*).
Bird 89, 1-15-39.

Size (increasing from A to D)	No. in singly infected cells	%	% large (C + D)	% small (A + B)
A	43	21.5		21.5
B	110	55.0		55.0
C	27	13.5	13.5	
D	20	10.0	10.0	
Totals	200	100.0	23.5	76.5

[1] Moshkovsky, S., and Poliakova, A., *Med. Parasit. and Parasitic Dis.*, 1934, **3**, 395. (In Russian; I have seen only the English abstract in *Trop. Dis. Bull.*, 1935, **32**, 409.)

tion at 1 P.M. the next day, one *dosis affectans* of atabrine having been given at midnight between the 2 examinations. Twenty-two birds survived long enough to be included in the record of this investigation; controls were injected with water alone.

10559

Microincineration of Active Smooth, Transitional and Skeletal Muscles.*

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The distribution of inorganic salts in the active and inactive smooth muscle of the intestine and the transitional muscle of the gizzard of birds and skeletal muscle is revealed by the technic of microincineration perfected by Scott.[1] He emphasized the distribution of ash in relatively inactive smooth and skeletal muscle by this method. He did not report in detail on the ash distribution in the active smooth, transitional, and skeletal muscles. This method of incinerating microscopic sections of tissue without disturbing the relationship of the mineral components was suggested by Liesegang[2] and developed by Policard.[3] The recent studies of Kruszynski[4] are on the topography of the mineral content in relatively resting muscle after microincineration.

The muscle is fixed for 24 hours in 9 parts of absolute alcohol and 1 part of neutral formalin. The mineral salts are neither increased nor decreased by this fixative. The tissue is completely dehydrated by several changes in absolute alcohol. The mineral salts remain intact by this method. It is then cleared in xylol, embedded in paraffin, and cut serially at 4 to 6 microns. Alternate sections are mounted by the usual method, and stained with hematoxylin and erythrosin, whereas, the intervening sections are mounted with liquid petrolatum to

* These investigations were carried out with the aid of a grant for Research to the Department of Anatomy, Marquette University School of Medicine, by the Committee on Scientific Research of the American Medical Association.

1 Scott, G. H., *Comp. Rend. Acad. Sci.*, 1930, **190**, 1073 and 1323; *Bull. Hist. app.*, 1930, **7**, 251; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 349; *Am. J. Anat.*, 1933, **53**, 243.

2 Liesegang, R. E., *Biochem. Z.*, 1910, **28**, 413.

3 Policard, A., *Protoplasma*, 1929, **7**, 464.

4 Kruszynski, J., *Z. f. Zellforsch. und Mikros. Anat.*, 1938, **28**, 35.

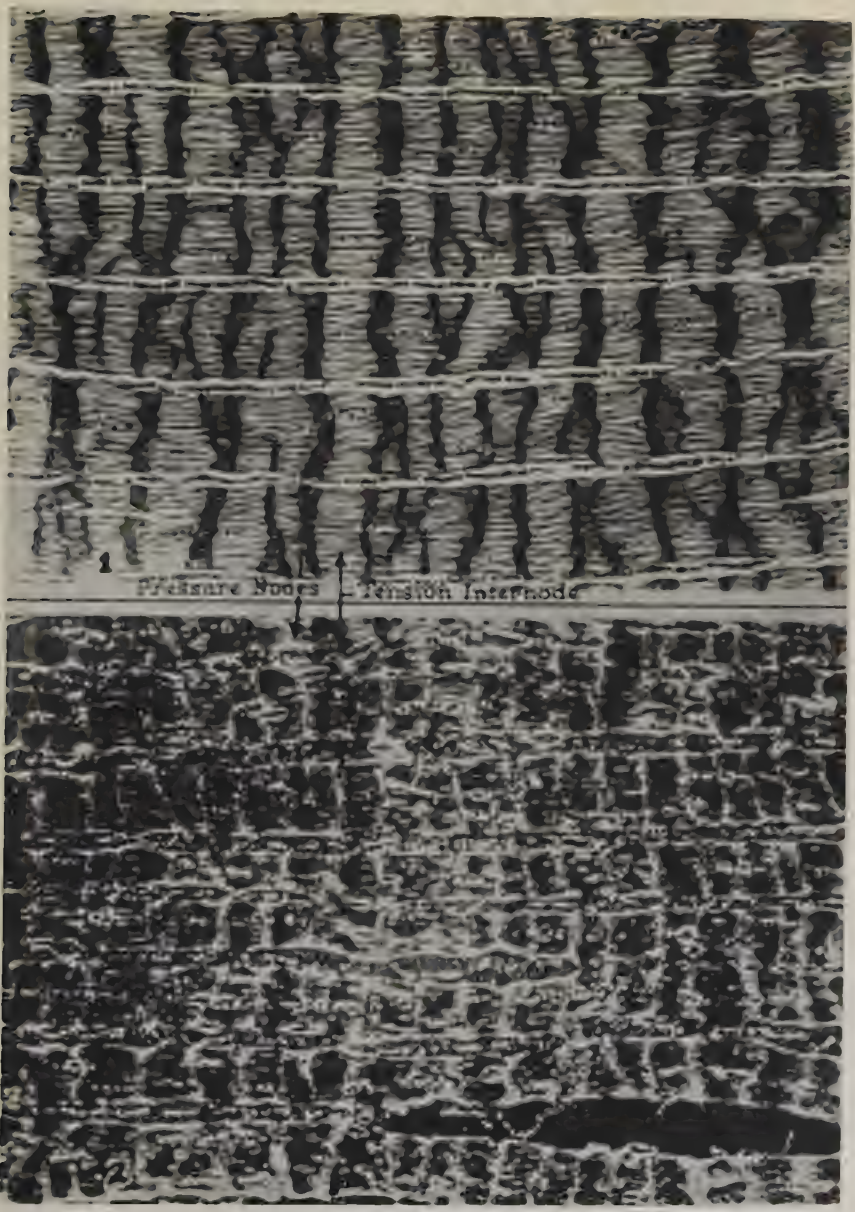
spread the tissue evenly. After the section is flat the excess liquid petrolatum is removed carefully by smooth blotting paper. The intervening sections are incinerated in a closed electric furnace at varying temperatures from 400° to 650°C.

The temperature is raised 50°C every 10 minutes until the highest temperature is reached. Sudden elevation of temperature with an excess of liquid petrolatum underlying the paraffin section results in explosive distortions due to sudden thermal agitation. The slide is cooled slowly over a period of 8 to 12 hours and the incinerated section is then covered with a number 0 cover glass around the edges of which melted paraffin is applied.

The inorganic salts that remain on the slide after microincineration of resting muscle are longitudinally distributed in the inactive cytoplasmic strands of the intestinal and gizzard muscles. In the active muscles there is a transverse orientation and concentration of the inorganic salts in the nodes of the contraction compression waves (Figs. 1 and 2). In the tension internodal spaces, between the pressure nodes, there are either less inorganic salts than in the nodes or in certain places almost a complete absence of detectable mineral salts. Iron oxide, calcium oxide, and silicates are identified in the inorganic deposit.

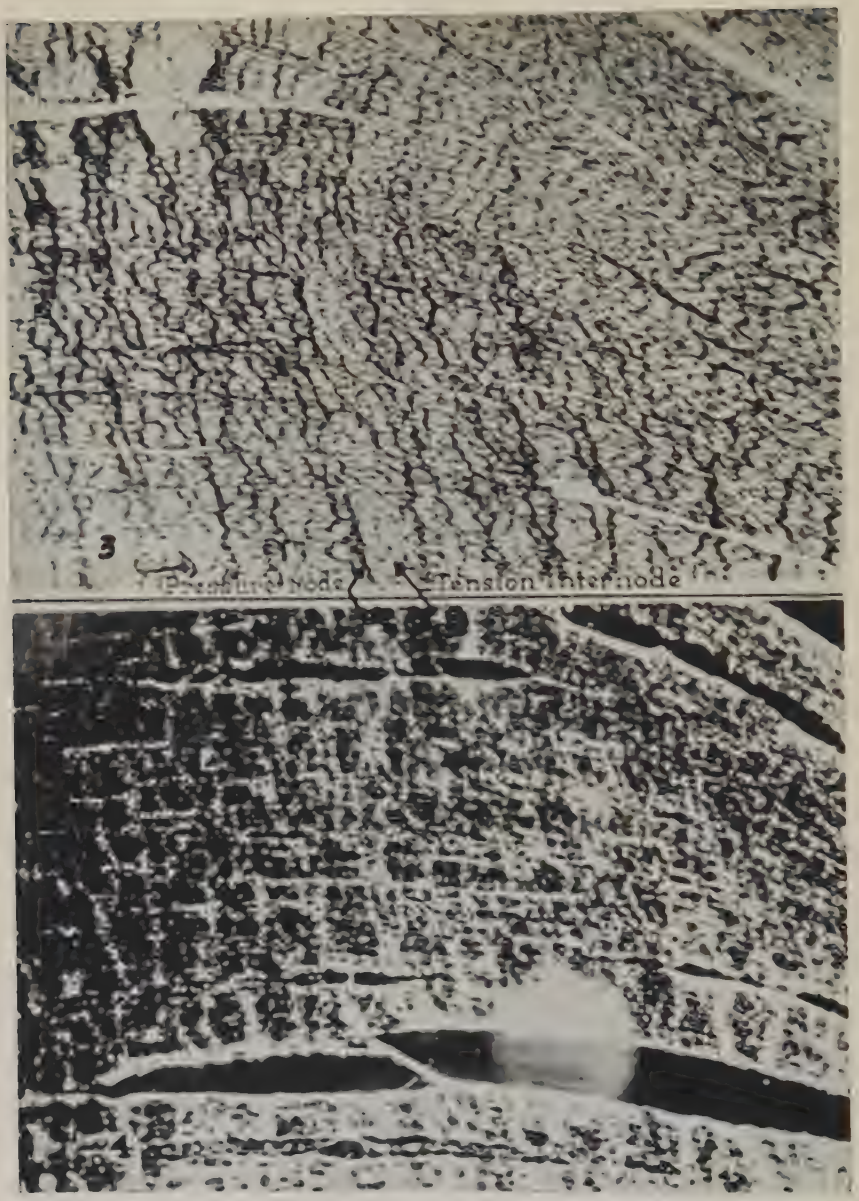
Freshly excised and ligated intestinal and uterine segments of the guinea pig one inch long and pieces of gizzard placed in Locke's solution at 42°C for 5 seconds and then 0°C for 3 minutes, followed by quick fixation, show multiplication in number of the contraction compression waves. The transversely placed nodal stripes composed of fine deposits of white and yellow-red ash are either regular or irregular in distribution. The intervening internodal space is almost clear of mineral residue. By comparing the alternate ashed and intervening stained serial sections it is apparent that the dense deposit of striped ash corresponds to the densely stained condensed cytoplasmic node in which the nuclei are rounded, compressed and densely stained. The rarefied internode in which the nuclei are elongated and the cytoplasm poorly stained corresponds to the relatively ash-free transverse band which alternates with the ash-bearing band. The granular ash that does remain in the internode is serially placed parallel to the long axis of the muscle fiber.

Phase differences in the contraction compression waves are detected by the variable degree of condensation and width of the ash bands at the nodes. In certain places the ash stripe forms either a uniformly dense, narrow, or broad band; in others there is an intervening relatively ash-free band bounded by parallel rows of a granular mineral deposit. In still other regions there is a central dense ash-



FIGS. 1 AND 2.

Stained section of active gizzard muscle of pigeon, Fig. 1. Compression nodes dark, tension internodes light. Microincinerated section, Fig. 2, ash-bearing nodes light. × 125.



FIGS. 3 AND 4.

Stained section of active gizzard muscle of pigeon, Fig. 3. Closely spaced dark nodes. Microincinerated section, Fig. 4, ash-bearing nodes light. × 150.

bearing band bounded on both sides by ash-free bands. All gradations of broad, widely spaced nodes 20 to 60 microns apart (Figs. 1 and 2) and narrow, finely spaced ones 3 to 20 microns apart (Figs. 3 and 4) are found by varying the intensity of the sudden application of heat, within physiologic limits, to the muscle prior to fixation.

Serial contraction compression waves in skeletal muscle fibers of the frog may be induced by either sudden elevations in temperature from 20°C to 37°C for 5 seconds or depression to 5°C, quickly followed by fixation. There is a nodal ash-bearing band composed of finely spaced and narrow Q striae alternating with an internodal band composed of diminished ash and coarse widely spaced Q striae. In skeletal muscle fibers of the frog the nuclei are compressed in the contraction node and elongated in the internode of the contraction compression waves. Scott[1] has previously demonstrated that the Q band, and Z band, when present, are ash-bearing and that the J band has diminution of mineral salts in skeletal muscle. This observation is confirmed.

In summary it may be said that the distribution of the mineral salts in the contraction compression waves induced by heat, within physiologic limits, within smooth, transitional and skeletal muscle is the same. There is a residue of ash-bearing node and a relatively ash-free internode. This is a mineral skeleton of active muscle free of organic matter. This distribution of mineral ash in active muscle fibers with serial nodes and internodes appears to correspond to the distribution of lycopodium powder when standing compression waves are produced in Kundt's tube, and to that of sand on glass slides by ultrasonic waves emanating from a crystal oscillator, demonstrated by Carey.[5] The powder and sand are aggregated in the pressure node and relatively absent in the tension internode when standing pressure waves are produced in the glass tube.

[5] Carey, Eben J., *Am. J. Anat.*, 1936, **59**, 175.

10560

Sucrose and Glucose Tolerance in Depancreatized Dogs.

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It is ordinarily assumed that sucrose in the diet of the diabetic produces exactly, gram for gram, the same effects as glucose. Thus in figuring the CHO content of a diet one makes no distinction between these two sugars. The work to be reported in this paper was instituted to determine whether this assumption is valid or not. The method used was essentially that of determining the comparative amounts of the two sugars that have to be administered to maintain a constant normal blood sugar in a depancreatized dog after the same amounts of insulin.[1]

On the morning of the experiment 2 or so units of insulin* were given intravenously; the purpose of this was to bring the blood sugar level to normal. Five or 6 hours after this first injection, by which time its activity had largely worn off but when the blood sugar was within normal limits, the determination proper was started.

Insulin for the determination proper was either given in one large dose (10 units), or by constant intravenous injection at a rate of about one unit per hour. Glucose and sucrose were given in amounts just sufficient to balance the activity of the insulin; in other words, to keep the blood sugar at a constant normal level. Blood sugar determinations were run every half hour in order to guide the adjustments in the amounts of sugar administered. Glucose was given either intravenously or by mouth, sucrose always by mouth.

Table I gives the results of the experiment. These figures show clearly that considerably greater amounts of sucrose than glucose can be taken with the same amounts of insulin. A possible cause of the difference between the two sugars might lie in the difference of absorption rates of them by the intestine. Since sucrose must first be inverted by digestive ferments, it might be claimed that its absorption is delayed; that it does not get into the circulation soon after feeding by mouth. A large portion of what is eaten might remain for some hours in the intestine, really piling up there and not acting to balance the insulin in the body. We have

[1] Greeley, P. O., Bergman, H. C., Tyler, D. B., and Drury, D. R., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 121.

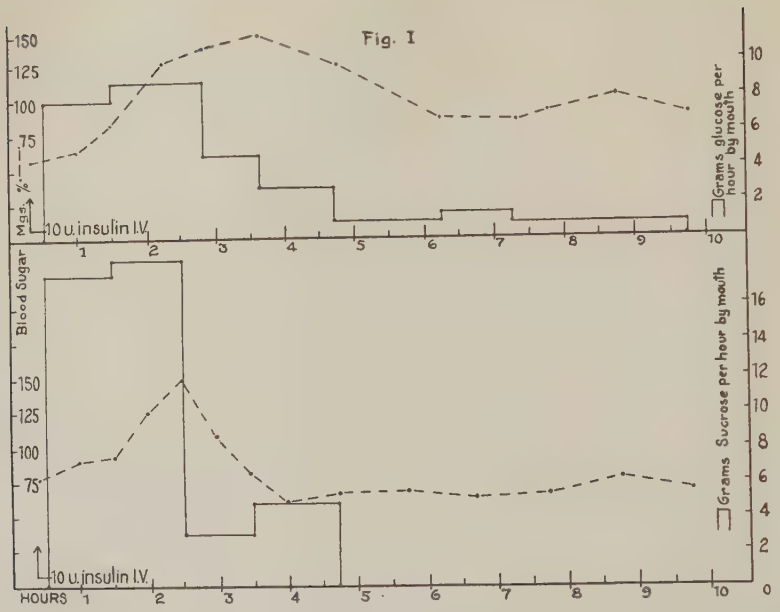
* The insulin used in this work was contributed by the Eli Lilly Company.

TABLE I.

Dog and date	Insulin per hr	Duration of run, hr	Type of sugar	Route	Total amt sugar given, g
	Results with Continuous Injection of Insulin.				
P 3-25-36	1.03	8	glucose	I.V.	30.5
P 4-20-36	1.03	8	sucrose	*Per os*	75
B 2-14-36	1.12	6.5	glucose	I.V.	31
B 3-14-36	1.12	6.5	sucrose	*Per os*	80
B 1-31-36	1.12	6.5	"	"	134
T 4-4-36	1.09	9	glucose	"	48
T 3-18-36	1.09	9	sucrose	"	96
T 3-21-36	1.09	9	"	"	94
	Results with One Large Dose (10 units) I.V.				
T 5-16-36		9	glucose	*Per os*	32
T 5-4-36		9	sucrose	"	53
R 3-14-39		7	glucose	"	10
R 3-31-39		7	sucrose	"	22

much evidence to present against this view. In the experiments in which insulin was given by constant injection, if the sucrose fed during the first hours were not rapidly absorbed it would pile up in the intestine and less and less would have to be given to balance the insulin as the experiment progressed. This was not the case, however. It was found that either a constant amount had to be given, or that this had to be increased somewhat as the experiment progressed, and behaved just like glucose in this respect. In one sucrose experiment, the feeding was stopped after 9 hours in order to see if there was enough sugar in the intestine to keep the blood sugar up. At the time of the last feeding the blood sugar was 115, after half an hour it was 100, and after one hour it was 71. This indicates that a half hour after the last feeding very little sucrose remained in the intestine.

Fig. 1 illustrates another type of experiment which indicates the same thing. This shows the results on the same dog on 2 different days after the injection of 10 units of insulin. In the upper chart, the insulin was balanced with glucose by mouth. In the sucrose experiment (lower figure), a large amount was fed the first 2 hours so that the blood sugar rose somewhat. The amount was then greatly diminished, the blood sugar promptly fell and remained at hypoglycemic levels for the rest of the run. If any appreciable amount of sucrose had remained in the intestine after the second hour it would have been absorbed in corresponding amounts at this time, and the blood sugar level would have continued to rise as it did during this period in the upper chart. Likewise, if any large amount had remained in the intestine after the fifth hour, it would have



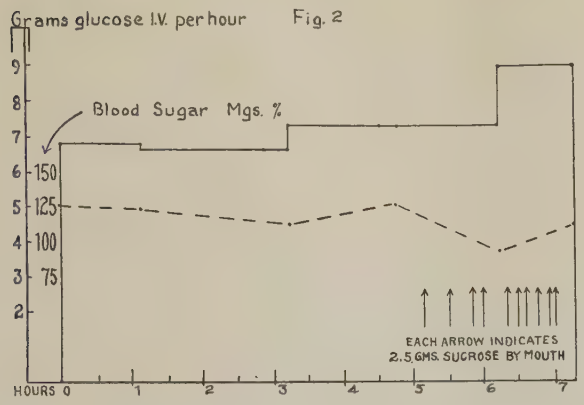
Blood sugar curves after administration of 10 units insulin. The blood sugar had been brought down by a small I.V. dose of insulin given 4 hours previously. In the upper chart the insulin activity is balanced by glucose, in the lower by sucrose. Comparison of the 2 charts shows that the large amount of sucrose given in the first 2 hours must have been absorbed very rapidly and did not leave a residue in the intestine to be absorbed in the later hours.

been absorbed in amounts sufficient to raise the blood sugar from hypoglycemic levels since, as may be seen from the upper chart, the blood sugar was kept at a normal constant level by giving such a small amount as one gram per hour.

In the case of B 3-14-36, the animal was put in a metabolism cage at the end of the experiment. Although it had taken 80 g of sucrose during the day (compared with 40 for glucose) the urine passed during the next 12 hours contained only 1.9 g glucose, an amount readily attributable to the fact that the animal had not received the fasting basal insulin during this time.[2] If the difference between glucose and sucrose were to be explained by the slow absorption of the latter and if in this experiment the 40 g extra sucrose were still in the intestine at the end of the run, it would have been absorbed during the next 12 hours and since the animal received no insulin during this time it would have been flooded with sugar.

Fig. 2 shows the effect of sucrose feeding superimposed upon an

2 Greeley, P. O., *Am. J. Physiol.*, 1937, **120**, 345.



Shows effect of feeding sucrose to a depancreatized dog in which constant intravenous insulin is balanced by constant intravenous glucose. The animal received 1.12 units insulin per hour throughout. The injection of insulin at this rate and of the balancing glucose was started 5 hours before the 0 hour of the chart.

animal in which insulin activity is balanced with intravenous glucose. This animal received 1.12 units insulin per hour throughout the experiment. During the first 5 hours the insulin activity is balanced by intravenous injection of 7 g glucose per hour. Then during a period of an hour and a half this rate is maintained, but in addition 10 g of sucrose is given by mouth. The blood sugar showed a sharp drop. Even with an increase in the rate of intravenous injection to 9 g of glucose per hour with 15 g sucrose by mouth the blood sugar does not rise to the level it was when the sucrose feeding was started. The urine passed during the next 9 hours contained only 0.7 g of glucose, an amount again attributable to the fact that the dog did not receive its fasting basal insulin during this time.

Conclusion. Definitely more sucrose than glucose can be tolerated in depancreatized dogs with the same dose of insulin.

10561 P

Origin of Fasting Ketosis in the Rat Following a Diet Low in Choline and Protein.

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When rats are fasted they develop a ketonemia and frequently it is high enough to produce a measurable ketonuria. The height of the ketosis is determined by many factors. When the diet is low in choline and in protein the liver becomes fatty[1] and on subsequent fasting there is a very substantial ketonuria.[2, 3] It seemed probable that this was incident to the fatty liver. However, since we have been unable to obtain a good correlation between the degree of ketosis and the amount of fat in the liver we have examined the factors which determine the extent of the fasting ketosis in question.

Deuel, *et al.*,[4] have found that choline administered during the period of fasting does not appreciably affect the ketosis of fasting fatty liver rats. We have confirmed this observation. Neither does the addition of choline to the diet prior to fasting, although it prevents the accumulation of fat in the liver, influence the subsequent ketosis. The only possibility left is that the ketosis of fasting rats is determined by their protein intake prior to fasting. This has been demonstrated in diets containing variable concentrations of casein, all containing 0.5% choline hydrochloride which insured livers low in fat when the fasting was commenced. The "0" protein diet was composed of sucrose 60, cod liver oil 2.5, Crisco 30, Standard Salt Mixture (Osborne and Mendel) 5, and Yeast Extract (Vitamin B Powder, The Harris Laboratories) 2. The other diets contained 5, 15 and 30% of casein respectively at the expense of the sucrose. Each group consisted of 5 adult male rats with an average body weight of 324 g. They had been receiving the special diets for 10 days before fasting was commenced. The protein intake tabulated per rat per day is the average of the 10-day period of feeding.

The data of a typical experiment recorded in Table I show very clearly the influence of the protein intake preceding a period of fasting upon the degree of fasting ketosis. Our ketonuria data as a

1 Best, C. H., and Wilkinson, H., *Biochem. J.*, 1935, **29**, 2651.

2 MacKay, E. M., *Am. J. Physiol.*, 1937, **120**, 361.

3 MacKay, E. M., Sherrill, J. W., and Barnes, R. H., *J. Clin. Invest.*, 1939, **18**, 301.

4 Deuel, H. J., Jr., Murray, S., Hallman, L. F., and Tyler, D. B., *J. Biol. Chem.*, 1937, **120**, 277.

TABLE I.
Ketone Bodies Excreted in the Urine per Rat per Day.

Protein intake prior to fasting g/rat/day	Day of Fasting				
	1	2	3	4	5
0	2	38	63	54	22
0.53	3	14	48	48	20
1.50	1	16	16	24	14
2.80	0	3	5	8	2

measure of ketosis is supported by the blood ketone levels which for the sake of brevity have not been included here.

In fasting rats the ketosis really develops after the glycogen stores are depleted at the beginning of fasting. The ketosis then might be dependent upon the antiketogenic action of the amount of "stored" protein now available for catabolism. However, nitrogen excretion figures do not support such a supposition.

10562

Basal Metabolism of 38 American-born Male Japanese University Students.

FRANCIS M. BALDWIN AND CHARLES K. FUJISAKI.

From the Physiological Laboratories of the University of Southern California, Los Angeles, California.

The effect of racial influence on the basal metabolism of Orientals, particularly the Japanese, has been debated for a long time. MacLeod, Crofts and Benedict[1] believe that there is an appreciable racial difference between the Americans and the Orientals, while Okada, Sakurai and Kameda[2] and other Japanese investigators deny its existence in the Japanese when they are compared with the classical American prediction standards. It is not the purpose of the present study to confirm or refute either one of these views, but the final results seem to indicate that there is a retention of racial characteristics in respect to the basal metabolism of Japanese. Whether or not this factor of race is influenced by differences in diet* and climate

1 MacLeod, G., Crofts, E. E., and Benedict, F. G., *Am. J. Physiol.*, 1925, **73**, 449.

2 Okada, S., Sakurai, E., and Kameda, T., *Arch. Int. Med.*, 1926, **38**, 590.

* While no attempt was made to control the dietary of the subjects, it is safe to say that the majority consumed two typically American meals (breakfast, lunch) consisting of cereals, milk, bread, and other essentials. The evening dinner meal was typically Japanese, the essential difference being the substitution of rice for bread in the American dietary.

TABLE 1.

Average* B.M.R. of a Group of Normal American-Born Male Japanese University Students.

Subject	Surf. area, m^2	Oral temp., °F	Rate per min. Pulse	Resp.	O_2 cc	†Cals./kg/24 hr	Deviations from Prediction Stds. Sanborn	Aub-DuBois	Harris-Benedict
1 S.H.	1.66	97.3	64	8	267	32.4	+13.6	+17.9	+17.8
2 G.H.	1.59	97.5	64	12	249	31.8	+ 8.7	+10.5	+15.5
3 Y.O.	1.62	98.1	55	9	248	30.3	+ 7.6	+12.4	+12.9
4 A.M.	1.63	97.6	63	11	248	28.5	+ 6.6	+11.1	+10.3
5 A.Y.	1.66	97.8	71	14	249	27.4	+ 4.7	+10.0	+ 7.5
6 C.O.	1.93	97.5	64	10	269	22.9	+ 0.4	+ 2.3	+ 0.1
7 L.I.	1.76	97.4	64	9	245	27.9	— 1.2	+ 2.1	+ 2.2
8 T.S.	1.72	97.3	65	7	238	26.8	— 1.4	+ 1.5	+ 2.2
9 E.T.	1.66	97.6	70	12	232	29.6	— 1.7	— 1.2	+ 4.7
10 H.N.	1.65	97.5	60	11	236	26.3	— 2.5	+ 0.9	+ 2.1
11 H.T.	1.67	97.0	52	9	237	23.5	— 3.2	+ 0.0	+ 1.9
12 S.O.	1.77	97.2	81	8	239	25.2	— 4.1	— 0.1	+ 1.3
13 S.N.	1.65	97.5	73	13	222	26.1	— 6.2	— 1.4	— 2.5
14 K.T.	1.66	97.5	66	13	215	26.1	— 6.3	— 5.1	— 3.3
15 F.O.	1.67	97.2	58	9	231	27.3	— 6.5	— 2.2	— 3.4
16 J.O.	1.64	96.5	57	13	216	23.8	— 6.5	— 3.5	— 5.0
17 M.N.	1.72	97.6	59	11	224	26.1	— 6.7	— 4.5	— 5.0
18 E.S.	1.75	97.8	61	11	225	23.9	— 8.1	— 5.6	— 6.2
19 H.K.	1.69	97.2	61	9	226	25.6	— 8.5	— 5.4	— 5.4
20 A.S.	1.66	98.2	64	20	205	24.3	— 8.6	— 9.4	— 6.6
21 J.Y.	1.65	97.7	56	12	212	25.7	— 9.6	— 6.1	— 5.1
22 T.N.	1.70	96.8	55	10	216	24.5	—10.0	— 6.1	— 6.5
23 T.K.	1.89	98.1	64	6	237	22.7	—10.6	— 8.1	— 8.6
24 F.M.	1.79	97.8	61	7	219	22.4	—11.3	—11.1	— 9.8
25 N.K.	1.74	97.0	75	10	223	24.9	—11.4	— 9.5	— 7.0
26 M.H.	1.56	97.6	65	12	196	25.6	—11.7	— 7.4	— 8.1
27 S.A.	1.54	97.0	67	14	191	24.8	—12.0	— 9.1	— 8.2
28 N.N.	1.78	97.4	53	15	227	23.9	—12.3	—10.2	— 8.0
29 G.N.	1.76	97.7	58	7	225	25.5	—12.6	— 6.8	— 7.6
30 R.T.	1.70	97.8	54	13	216	24.1	—12.7	—10.0	— 7.8
31 T.I.	1.86	97.3	60	9	255	24.7	—13.1	— 7.7	— 3.9
32 G.T.	1.66	97.4	68	10	208	24.9	—13.3	—10.0	— 7.5
33 J.S.	1.67	96.7	61	10	213	24.5	—13.9	—10.2	— 7.8
34 R.M.	1.56	97.4	58	6	192	23.7	—13.9	— 9.6	—16.0
35 T.Y.	1.74	96.5	54	9	212	24.8	—15.2	—10.6	—10.3
36 Y.F.	1.69	97.4	64	15	202	22.7	—16.2	—12.1	—11.4
37 M.I.	1.53	97.7	61	12	174	23.1	—16.7	—16.7	—14.3
38 R.H.	1.61	97.1	62	7	175	21.9	—22.8	—20.5	—22.5
Grand Average	1.68	97.4	62	11	224	25.6	— 7.1%	— 4.1%	— 3.4%

*As advocated by DuBois the average of two lowest results from three trials on different days was made.

†Heat production expressed in calories.

will not be definitely known until more experimental evidences are brought forth.

The basal metabolism of 38 apparently normal American-born men, college students attending 2 large universities† in California was measured indirectly in terms of oxygen consumption by a Sanborn apparatus. These subjects were descendants of Japanese immigrants who had been exposed to Western civilization for several decades. The average metabolic rate for the entire group according to the Harris-Benedict prediction normals is –3.4% and according to the Aub-DuBois standards the mean deviation is –4.1%. This result is slightly lower in regards to the Sanborn prediction tables, being –7.1%. The average physical characteristics of the entire 38 subjects are as follows: age, 20.6 years; weight, 60.9 kg; height, 167.6 cm; and surface area according to height-weight of DuBois,[3, 4] 1.68 square meters.

The final results of the present investigation are remarkably in accordance with the average basal metabolic rate and physical characteristics of 38 Hawaiian-born Japanese college and high school students who were studied by Miller and Benedict.[5] They are also in a close agreement with those measurements of Okada and his associates on a group of 42 medical students in Japan, as shown in Table II. Although there are differences in nutritional and climatological factors of these 3 geographical localities, the final results of average basal metabolic rates are remarkably within a narrow range of 4%. This fact tends to support the conception that there is a

TABLE II.
Average Metabolic Rates of Various Japanese Groups.

Investigators concerned	Subjects No. and Local.	Deviations from tables Aub-DuBois	Harris-Benedict
		%	%
Okada *et al.*	42 (Japan)	—2.0	—0.2
Miller-Benedict	38 (Hawaii)	—3.9	—2.8
Baldwin-Fujisaki	38 (America)	—4.1	—3.4
	Avg	—3.3	—2.1

† University of California at Berkeley, and the University of Southern California at Los Angeles.

3 DuBois, Eugene F., *Basal Metabolism in Health and Disease*, 3d ed., Philadelphia, Lea and Febiger, 1936, 494 pp.

4 DuBois, Eugene F., *J. Nutrition*, 1930, **3**, 217, 331.

5 Miller, C. D., and Benedict, F. G., Basal Metabolism of Normal Young Men and Women of Various Races in Hawaii and Basal Metabolism of Samoan Race, *The University of Hawaii Research Publications*, No. 15, Honolulu, 1937, 77 pp.

hereditary retention of racial characteristics among the Japanese as far as their basal metabolism is concerned.

Summary. 1. The average metabolic rate of 38 American-born male Japanese university students is –3.4% according to the Harris-Benedict and –4.1% according to the Aub-DuBois prediction standards. 2. The results of the present study are remarkably in accordance with those data of Miller and Benedict in Hawaii and of Okada, *et al.*, in Japan. 3. A comparison of data from 3 different geographical localities seems to support the conception that racial characteristics are retained hereditarily as far as the basal metabolism of Japanese is concerned.

10563

High Fat and High Carbohydrate Diets That Can Be Fed to Rats by Stomach Tube.*

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In order to maintain body weight in hypophysectomized rats it was found necessary to develop diets which could be fed by stomach tube in amounts sufficient for adequate nutrition. Two examples of satisfactory diets follow:

High Carbohydrate diet	g
Dried egg albumin†	15
Corn starch	42
Dextrin (from corn)	21
Cane-sugar	21
Osborne-Mendel salt mixture	4
Activated charcoal	5
Cellu-Flour	5
Vitamins B and D	
Enough water to make 125 cc	

High Fat Diet	g
Dried egg albumin†	15
Melted butterfat‡	37.8
Osborne-Mendel salt mixture	4
Cellu-Flour	10
Vitamins B and D	
Enough water to make 125 cc	

The salt mixture, Cellu-Flour, and casein should pass through a 100 mesh screen. The dry ingredients are mixed together and the melted butter or cream stirred in. Finally, small amounts of water are added until the required volume is reached and the whole then

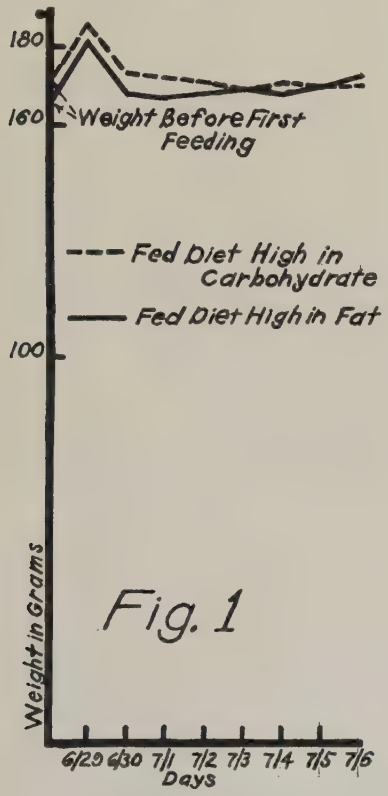
* This work was aided by grant No. 453 of the Committee on Scientific Research of the American Medical Association.

† 14.5 g of casein and 0.5 g of gelatin may be used in place of the albumin.

‡ 63 cc of 60% cream may be substituted.

thoroughly mixed. The mixture is passed through an homogenizer until uniformly creamy.

For administration the distal 5″ of a No. 8 French rubber catheter is fitted to a hypodermic syringe and used as a stomach tube. The piece of catheter is best attached by sliding its proximal end over a large sized needle filed off square about ½″ from its hub or by the fitting shown in Fig. 2. The syringe is next filled with the proper amount of food. The tube is then wetted and passed down the rat's esophagus with a to and fro rotary movement while an assistant holds the rat's mouth open by a forceps placed back of its incisors and spread quite widely. The tube starts more easily if the rat's



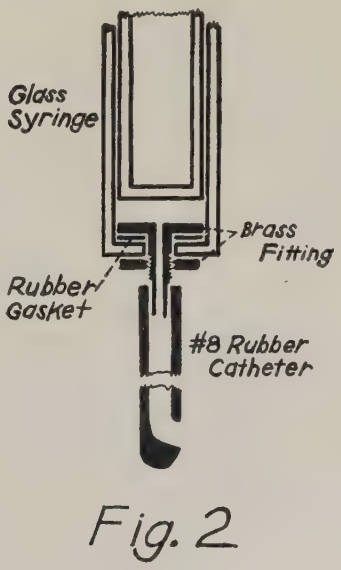


FIG. 1.

The average daily weights of six hypophysectomized rats fed a diet high in carbohydrates and nine hypophysectomized rats fed a diet high in fat. The rats were weighed each day before the last feeding. The peak on 6/29 is probably due to the addition of the food fed to that already in the rat's gastrointestinal tract before the feedings were started.

FIG. 2.

Fitting for attaching a catheter to an all glass syringe. The nib of an ordinary glass syringe can be broken off and the hole enlarged to the proper size with a three-cornered file. Certain brands of syringes will not function with these diets.

head is kept well flexed on its neck. The tube seldom enters the trachea but if such an accident occurs it can be recognized at once since not nearly all of the tube can be passed. Under such circumstances it should be gently withdrawn without delay.

With practice one person can learn to hold the rat with the left hand and pass the tube with the right. The thumb of the left hand is pressed against the rat's mandible to prevent his incisors from occluding and puncturing the catheter. With practice one can feed a rat in about 2 minutes. In most instances, the amount fed should be proportional to the rat's surface area. For young adult rats the amount required will be about 5 cc for each of 3 daily feedings. Diets which are inconveniently stiff may be diluted slightly and given in proportionately larger amounts.

The following example illustrates the use of these diets: A group of 6 hypophysectomized rats averaging 170 g in weight and fed 4.3 cc of the high carbohydrate diet per day per 100 sq cm of body surface showed an average change in weight of less than 1 g after 8 days of feeding. A similar group of 9 hypophysectomized rats fed a corresponding amount of the high fat diet gained an average of 9 g apiece during the same period of feeding. The average weights of the 2 groups are plotted in Fig. 1. The daily feeding was given in 3 equal portions at least 5 or 6 hours apart. In addition the animals were allowed to drink Rubin-Krick solution[1] *ad libitum*. None of the rats showed signs of esophageal irritation but 3 rats on the high carbohydrate diet and one rat on the high fat diet died from other causes connected with hypophysectomy and are not included in the data. Normal controls were equally well maintained on the same amounts of the diets.

[1] Rubin, M. J., and Krick, E. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 228.

10564 P

Effect of Localized Increased Temperatures on a Frog Egg (*Hyla regilla*)

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It is well known that the amphibian organizer center (dorsal blastoporal lip) induces a neural plate in any presumptive ectoderm with which it may come in contact. It has been claimed that neural plates may also be induced in presumptive ectoderm by application of a thermal gradient (Gilchrist,[1] Castelnuovo[2]) as would be expected on the basis of Child's[3] gradient theory. Our experiments on the tree frog *(Hyla regilla)* have not thus far substantiated the latter work.

The apparatus used, we believe, is easily the most accurate yet applied to this study of the effects of thermal gradients on amphibian eggs. The thermodes used were made of 4 coils of platinum wire 1/500 of an inch in diameter, insulated with a thin coat of lacquer, and heated with current from a storage battery. The temperature could be regulated by a resistance box. The coils of platinum wire were carefully placed against the upper surface of the egg by means of a micro-manipulator set. It was possible to observe the eggs constantly during the period of heating. The eggs were also vitally stained where the thermode was placed, in order to be certain that no movement took place.

The temperature applied to the egg surface was ascertained directly by the use of a micro-thermocouple and galvanometer.

The thermal death-point of the cells was found to lie at about 36-38°C. Our experiments to date have been concerned with the application of temperatures close to the thermal death-point (27-36°C). On the basis of the physiological gradient theory of Child, these high temperatures ought to be most effective in inducing secondary neural structures.

Examination of the external surfaces of the heated eggs has thus far revealed no case of clear neural induction. The heated portion is usually more advanced in development than the rest of the egg. Thus, in one case in which the ectoderm of the face region was heated, the typical prominences of the suckers were present anteriorly

1 Gilchrist, F. G., *Physiol. Zool.*, 1928, **1**, 231.

2 Caltelnuovo, 1932, quoted in Dalcq, A., and Pasteels, J., *Bull. de l'Acad. royale de Médecine de Belgique*, 1938, VIe série, tome 3, 261.

3 Child, C. M., *Protoplasma*, 1928, **5**, 447.

while the neural folds were not yet closed in the middle and posterior parts of the body. One entire neural fold may arise on the heated side before any fold appears on the opposite side or in the control (non-heated) eggs.

The specimens we have thus far examined in sections show no clear neural induction. However, there are ectodermal thickenings and in some cases the heated ectodermal cells are columnar in form, whereas non-heated ectoderm of the same egg is composed of low cuboidal or flattened cells. Whether this may represent a slight attempt at neural induction, is doubtful at present. We wish to emphasize that the implantation of organizer material in *Hyla regilla* results in perfectly clear induction in almost 100% of the cases (Schechtman[4]).

10565 P

Experiments on Anus Formation in a Frog Egg.

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The anus arises in *Hyla regilla*, as in most vertebrates, as an ectodermal (proctodeal) pit which subsequently becomes perforated to form the anus. We have attempted to analyze the factors responsible for this differentiation.

The presumptive proctodeal ectoderm of the early gastrula has no capacity to form a proctodeal pit. This ectoderm was transplanted to the chest region, where it formed smooth epidermis. The anterior ectoderm (of the face and chest) were transplanted to a posterior position, just over the ventral blastoporal lip, and here developed into very clear proctodea. Evidently the ventral lip contains some factor or factors responsible for the appearance of the proctodeum, as was previously concluded.[1] When the ventral lip is implanted into the blastocoele of another early gastrula, the host ectoderm develops a proctodeum usually on the side or belly.

In 2 series of extirpation experiments the mesoderm or the entoderm of the ventral lip was removed from the early gastrula. In the absence of the entoderm a proctodeum forms in all cases. After re-

[4] Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 236.

[1] Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 236.

moval of the mesoderm most of the eggs fail to form a proctodeum, but a few develop a complete or rudimentary proctodeal pit. This we attribute to incomplete removal of the mesoderm.

Ventral lip mesoderm from the early neurula, in which the 3 germ layers are more clearly distinguishable, was implanted into the blastocoeles of early gastrulae. The mesoderm, taken from the region just under the slit-shaped blastopore, induces a clear proctodeum in the host ectoderm. The entoderm of the hind-gut region was implanted into other early gastrulae and was not capable of inducing a proctodeum. But the epidermis of the host lying just over the implant becomes perforated, exposing the implanted piece of hind-gut.

We attempted to delimit the proctodeum-inducing region by explanting various segments of the blastoporal lips of the early gastrula. The ventral and ventro-lateral lips develop proctodea in almost all cases. The lateral lips do so in some cases (exact percentage not yet available). Dorsal and dorso-lateral lips never form a proctodeum. The inductor thus must extend approximately over the entire ventral half of the mesodermal ring. However, when only the ventral lip is removed from an early gastrula, the 2 ventro-lateral and lateral lips remaining, the embryo fails to form a proctodeum. Evidently some sort of regulatory mechanism is at work. An interesting feature of these eggs is the consistent absence of the ventral fin of the tail.

Holtfreter[2] found that an anus was induced when chorda, neural tube, entoderm, and connective tissue were present in various combinations near the ectoderm. Proctodea were also present when no neural tube, entoderm, or chorda lay near the ectoderm, although apparently in all cases some connective tissue was present. Risley[3] has advanced evidence that connective tissue alone is capable of the induction; he believes it possible that the connective tissue receives its inductive capacities from the axial organs. Our work on *Hyla* makes it clear that, at least as concerns the proctodeum of the frog, there is no necessity to assume such a rôle of the axial organs. This follows from the fact that the ventral lip, which contains no chorda and is incapable of inducing a neural tube, can induce the proctodeum. Risley has also suggested that the caudal fin may be a necessary factor in anus formation. We have numerous cases in which a proctodeum was formed in the complete absence of a fin.

From the results given above we may suggest the following mechanism of anus formation: The ventral lip mesoderm induces the

2 Holtfreter, J., *Arch. f. Entw.-mech.*, 1936, **134**, 466.

3 Risley, P. L., *J. Exp. Zool.*, 1939, **80**, 113.

proctodeal pit in that portion of the ectoderm which happens to come into contact with it. The invagination of the pit brings it into contact with the entoderm of the hind-gut. The latter completes the process of anus formation by inducing the perforation of the proctodeal pit.

10566 P

Failure of "Vitamin K" Excess to Heal Encephalomalacia of Chicks.

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From the Department of Bacteriology and Public Health, University of Illinois College of Medicine, Chicago.

During the course of experimentation on chick rabies attention was drawn to the fact that most of the early literature did not consider the dietary factors necessary for normal nervous system development in the fowl. Pappenheimer, *et al.*,[1, 2] described the encephalomalacia present in experimental and normal flocks. This paper is concerned with the effects of a high "vitamin K"* level in an attempt to alter the encephalomalacia in chicks which is believed due to capillary alteration by some unknown factor missing from the diet.

Healthy day-old chicks were placed on deficiency diet No. 108,[1] "normal" diet No. 20[1] and controlled by a series on a ration similar to No. 20, but more satisfactory for growth. The chicks had no access to their feces. The alfalfa leaf meal extract (hexane) was homogenized in cream with the aid of a Jubilee type homogenizer and mixed with milk. Daily levels of 5 mg, 10 mg, 15 mg, and 20 mg were fed to the 4 series consisting of a total of 120 chicks on the deficiency diet.

Results. Six paralytic chicks occurred in a series of 30 on the deficiency diet without excess "vitamin K"; while 19 paralytic chicks occurred in the 4 series of the 120 chicks receiving the deficiency diet plus excess levels of "vitamin K." One paralytic chick recovered spontaneously while on the deficiency diet without excess "vitamin K" in contrast to 12 paralytic chicks receiving the excess "vitamin

1 Pappenheimer, A., and Goettsch, M., *J. Exp. Med.*, 1931, **53**, 11.

2 Goettsch, M., and Pappenheimer, A., *J. Biol. Chem.*, 1936, **114**, 693.

* The alfalfa leaf meal was generously contributed by Dr. W. Wenner, The Upjohn Company, Kalamazoo, Michigan. 1.0 cc of this extract is equal to 10 g in "vitamin K" activity of alfalfa meal.

K". There were no differences observed in the clinical and microscopic picture of the paralytic chicks on the deficiency diet alone or with excess levels of "vitamin K." The effects of high levels of "vitamin K" with experimental rations will be reported later.

Conclusion. Encephalomalacia of chicks is not prevented by the administration of a "vitamin K" excess.

10567

Effect of Normal and Renal Hypertensive Dog Plasmas on Surviving Arterial Rings.*

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From the Department of Physiology, College of Medicine, University of Illinois.

At the present time there is inconclusive evidence both for and against the presence of a pathogenetic pressor substance in the blood of dogs rendered hypertensive by the technic of Goldblatt. We have studied this question by determining the effect of citrated plasmas from normal and renal hypertensive dogs on the tonus of surviving beef arterial rings. Since citrated plasma from normal dogs contains a constrictor substance or substances, we looked for a possible quantitative difference between the tone-augmenting effects of the normal and hypertensive plasmas on the arterial rings.

The method employed was essentially that of Meyer[1] and Dale and Laidlaw.[2] Rings about 4 mm in diameter and 3 mm in width were obtained from the tertiary division of the superior mesenteric artery of freshly slaughtered beeves and stored for 24 to 72 hours in Locke's solution at 4°C. The arterial ring was then placed in a smooth muscle chamber of 8 cc capacity containing oxygenated Locke's solution maintained at 37°C by a constant temperature bath. The lever used magnified the tonus changes of the arterial ring eleven times. The initial tonus of the arterial segment was partially overcome by a stretching load of 2 g attached to the long arm of the lever at the same distance from the fulcrum as the attachment of the artery to the short arm. After the tonus level of the arterial ring had relaxed to an equilibrium level, usually in a period of one hour,

* This work was aided by a grant from the Graduate School Research Fund of the University of Illinois.

1 Meyer, O. B., *Z. f. Biol.*, 1906, **43**, 352.

2 Dale, H. H., and Laidlaw, P. P., *J. Pharm. and Exp. Therap.*, 1912, **4**, 75.

the preparation was ready for use, the 2 g weight being used as a lifting load.

The citrated plasmas were obtained from the femoral artery by mixing 1.5 cc of 5% sodium citrate with 18.5 cc of blood. Usually the plasmas were tested only on the same day although on 4 occasions they were again tested after 24 or 48 hours in the ice box at 4°C. Sixteen separate experiments were performed using arterial segments from a corresponding number of beeves. Two normal and 2 hypertensive plasmas were usually employed for each experiment. The suitability of the arterial ring for use was first demonstrated by its prompt vasoconstrictor response to a 1:5,000,000 solution of epinephrine HCl in Locke's solution. Five minutes later, the epinephrine solution was replaced, after washing, by Locke's solution with a resulting return of the ring to its original tonus level during the next 15 minutes. Subsequently the same procedure was used for the plasmas, alternating the normal and hypertensive plasmas. The plasmas were then retested in the same order and finally the epinephrine solution was repeated.

The plasmas from 6 normal and 6 renal hypertensive dogs were tested a total of 3 to 6 times at biweekly or monthly intervals. The plasmas of 3 of the hypertensive dogs were tested both before and after the production of renal hypertension by constriction of the renal arteries.

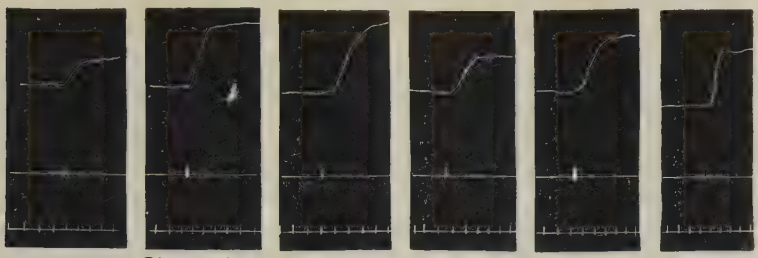
One-third of the relaxed arterial rings showed spontaneous rhythmic tonus changes in Locke's solution which have already been described.[3, 4] Fig. 1 illustrates a typical experiment with 2 normal and 2 hypertensive plasmas. The first normal plasma from a dog with a mean femoral blood pressure (measured by the method of Dameshek and Loman[5]) of 134 mm of Hg. produced an elevation of 21 mm in the writing point of the lever; the first hypertensive plasma from a dog with a blood pressure of 176 mm showed a rise of 23 mm; the second normal plasma from a dog with a pressure of 138 mm caused an increase of 12 mm; and the second hypertensive plasma from a dog with a pressure of 192 mm resulted in an increase of 18 mm. Immediate retesting of these plasmas on the same arterial ring showed the same relationships in vasoconstrictor effect, although the effect was reduced by about one-fourth in each case, undoubtedly due to the removal of some of the vasoconstrictor activity by the arterial ring. This reduction was an invariable finding in all of

[3] Janeway, T. C., Richardson, H. B., and Park, E. A., *Arch. Int. Med.*, 1918, **21**, 565.

[4] Wakerlin, G. E., and Bruner, H. D., *Arch. Int. Med.*, 1933, **52**, 57.

[5] Dameshek, W., and Loman, J., *Am. J. Physiol.*, 1932, **101**, 140.

Figure 1.
The effect of epinephrine and of normal and hypertensive plasmas on the beef arterial ring



1:5,000,000 epinephrine in Locke's solution | Plasma from normal dog with mean b.p. of 134 mm. | Plasma from hypertensive dog with mean b.p. of 176 mm. | Plasma from normal dog with mean b.p. of 138 mm. | Plasma from hypertensive dog with mean b.p. of 192 mm. | 1:5,000,000 epinephrine in Locke's solution

the experiments. The 1:5,000,000 solution of epinephrine produced a rise of 9 mm at the beginning of the experiment and a rise of 18 mm at the end. This increase in epinephrine effect after the plasmas was observed in each experiment and is probably ascribable to the sensitizing action of the plasma proteins.[6]

Subsequent comparisons of the plasmas from these 4 dogs yielded similar results. We were likewise unable to find any correlation between the tone-augmenting action of the plasmas and the blood pressure levels of the other 4 normal and 4 hypertensive dogs. Furthermore, there were no significant differences between the vasoconstrictor effects of the plasmas before and after the production of hypertension in the 3 dogs so studied. The detailed results are illus-

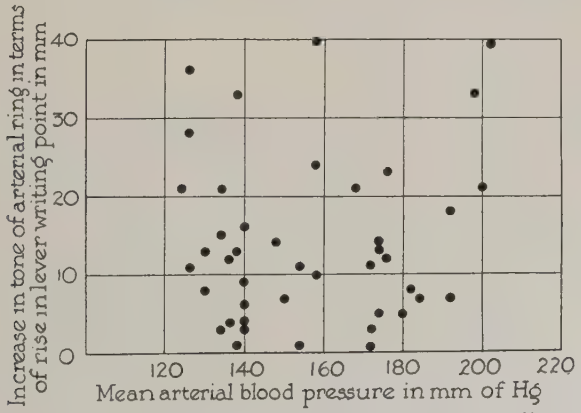


Fig. 2. The relation of the tone-augmenting effects of the plasmas of normal and hypertensive dogs to their mean femoral blood pressure

6 Freund, H., and Gottlieb, R., *Arch. exp. Path. u. Pharm.*, 1922, **93**, 92.

trated in Fig. 2, where the increases in tone of the arterial rings as measured by the rise of the lever writing point are plotted against the mean femoral blood pressures.

In the 4 experiments where the plasmas were again tested after 24 or 48 hours at 4°C, the vasoconstrictor activity of the plasmas was invariably somewhat more than doubled.

The absence of significant differences in the tone-augmenting property of the normal and hypertensive plasmas for the arterial rings is in agreement with the inability of Page,[7] Collins and Hoffbauer,[8] Prinzmetal, *et al.*,[9] and Heymans and Bouckaert[10] to demonstrate any increase in pressor substances in the blood of renal hypertensive dogs. On the other hand, the reports of Dicker,[11] Govaerts and Dicker,[12] and particularly Fasciolo, *et al.*,[13] suggest a significant increase in the pressor action of the blood of renal hypertensive dogs. The limitations of our method are such that positive results only would be conclusive. The most obvious shortcomings of the method employed are the following: The effects of citrated dog plasma on beef arterial rings *in vitro* are not necessarily analogous to the action of normal plasma on arterioles *in vivo*. The use of dog plasmas and beef arterial rings introduced an unknown factor of species difference. Furthermore, the effect of the possible pathogenetic pressor substance may have been overshadowed by the action of the vasoconstrictor substance present in citrated plasma. And lastly, the hypothetical pressor substance may be present in demonstrable quantities in the venous return from the ischemic kidney but not in the systemic blood. Our results, therefore, by no means rule out the possibility of such a pressor substance in experimental renal hypertension.

Conclusions. 1. The effects of citrated blood plasmas from 6 normal and 6 renal hypertensive dogs on the tonus of arterial segments from the mesenteric arteries of beeves were studied. 2. No significant differences were found in the vasoconstricting properties of the normal and hypertensive plasmas. 3. These results speak against the presence of a peripherally acting pathogenetic pressor substance in the blood of Goldblatt dogs but by no means rule out this possibility.

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7 Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 112.

8 Collins, D. A., and Hoffbauer, F. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 539.

9 Prinzmetal, M., Friedman, B., and Oppenheimer, E. T., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 493.

10 Heymans, C., and Bouckaert, J. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 94.

11 Dicker, E., *Comp. rend. Soc. de biol.*, 1936, **122**, 476.

12 Govaerts, P., and Dicker, E., *Compt. rend. Soc. de biol.*, 1936, **122**, 809.

13 Fasciolo, J. C., Houssay, B. A., and Taquini, A. C., *J. Physiol.*, 1938, **94**, 281.

10568 P

Human Sternal Marrow in Hyperthyroid and Myxedematous States.

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In a recent comprehensive review of observations made on the bone marrow in states of thyroid dysfunction Bomford[1] concludes that the scanty evidence available indicates a partial atrophy of the marrow in hypothyroidism. In hyperthyroidism there is first an increased activity, and then a scarcity of hemopoietic marrow cells. He believes that diminished oxygen requirements of all the tissues and consequent lack of stimulation to erythropoiesis, in hypothyroidism causes a quantitative hypoplasia of active marrow. This belief is based on postmortem findings in cretins[2–6] and studies of rabbit marrow after thyroidectomy.[7, 8] The hyperthyroid marrows were obtained from rabbits after the feeding of thyroid or injection of thyroxine.[9, 10]

I am reporting the results of studies of the aspirated sternal marrow of humans in "normal", hyperthyroid, and myxedematous states. The method[11] of obtaining and studying the marrow has been in use at the University of Illinois for over 20 months and to date over 700 aspirations have been done in a wide variety of conditions. The technic and apparatus used in the above method were recently reported in detail.[12]

Aspirated sternal marrow from 18 individuals with normal peripheral blood picture and no complaints other than a hernia in several, contained an average of 6.2% (ranging from 4 to 10%) of nucleated cells. Stained smears of these nucleated cells revealed

1 Bomford, R., *Quar. J. Med.*, 1938, **31**, 495.

2 Langhaus, T., *Virchow's Arch. f. path. Anat. u. Physiol.*, 1897, **149**, 155.

3 Maresch, R., *Z. f. Heilk*, 1898, **19**, 249.

4 Dieterle, T., *Virchow's Arch. f. path. Anat. u. Physiol.*, 1906, **184**, 56.

5 Stoccada, F., *Beit. z. path. Anat. u. z. allg. Path.*, 1915, **61**, 450.

6 Askanazy, M., *Sang.*, 1930, **4**, 1.

7 Tatum, A. L., *J. Exp. Med.*, 1913, **17**, 636.

8 Kunde, M. M., Green, M. F., and Burns, G., *Am. J. Physiol.*, 1931-2, **99**, 469.

9 Power, T. D., *Studies in Blood Formation*, 1934.

10 Lim, R. K. S., Sarkar, B. B., and Brown, J. P. H. G., *J. Path. and Bact.*, 1922, **25**, 228.

11 Schleicher, E. M., and Sharp, E. A., *J. Lab. and Clin. Med.*, 1937, **22**, 949.

12 Limarzi, L. R., *Ill. Med. J.*, 1939, **75**, 38.

that the ratio of myeloid to erythroid cells fell between 2:1 and 4:1.

Marrow taken in the same way from 12 individuals in a hyperthyroid state contained an average of 13.5% (7% to 22%) nucleated cells with a myeloid-erythroid ratio in the stained smears running from 5 to 1 to as high as 20 to 1. There was also an apparent marked increase in the megakaryocytes found in these smears when compared with the "normals".

Marrow taken from 7 myxedematous individuals contained an average of 2.4% nucleated cells (ranging from 1.5% to 4%) and the myeloid-erythroid ratio varied from 1:1 to 3:1.

The aspirated sternal marrow of 6 of the 7 in a myxedematous state was studied subsequent to the feeding of desiccated thyroid or injection of thyroxine and in all cases a marked rise in the percentage of nucleated cells in the marrow was found. This change varied in magnitude. There was a rise from 1.5% to 3% in 4 weeks in one case, and from 2% to 14% after 3½ months in another. This increase was accompanied by a rise in the basal metabolic rate and a fall in the blood cholesterol.

One individual with a thyrotoxicosis due to the ingestion of desiccated thyroid had 20% nucleated cells in the aspirated marrow when her basal metabolic rate was +150%.* This fell to 12% two months later, at which time her basal metabolic rate was +4%, and to 6% nucleated cells six months after the thyroid ingestion was stopped. Following sub-total thyroidectomy all marrows studied showed a less marked but consistent decrease.

Peripheral blood studies were done at the time of marrow aspiration in all cases. In the hyperthyroid individuals, a most interesting fact was that there was no reflection of the myeloid hyperplasia of the marrow in the peripheral blood. All 7 in the hypothyroid state were somewhat anemic. Five had an average red cell volume within normal limits, while one was macrocytic (106 cu microns), and one had a frank iron-deficiency anemia (67 cu microns).

Sternal marrow aspirated from individuals in a state of thyrotoxicosis shows a marked myeloid hyperplasia when compared to that of "normals". The average percentage of nucleated cells found in hyperthyroidism is over twice the "normal" average. This myeloid hyperplasia contrasts sharply with the erythroid type of most anemias, and in over 700 marrow biopsies, most closely resembles that found in chronic myelogenous leukemia. The myeloid hyperplasia of the sternal marrow is not reflected in the peripheral blood,

* Average of two tests done after rest and sedation. Patient was restless and these probably are not true basal rates.

as is usually the case in myelogenous leukemia. In the small number of cases studied the myeloid hyperplasia tends to return to "normal" when the thyrotoxicosis is removed.

Aspirated sternal marrow from individuals in a myxedematous state is markedly hypoplastic. Sufficient feeding of desiccated thyroid or injection of thyroxine causes an increase in the nucleated cells of this marrow to "normal".

These findings indicate that the thyroid secretion has a regulatory effect on quantity and quality of the bone marrow, as evidenced by differences found in the quality and quantity of aspirated sternal marrow.

10569 P

The Adrenals in Experimental Hypertension.

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From the Laboratory of Experimental Endocrinology, School of Medicine, University of Pittsburgh, and the Physiological Laboratory, University of Chicago.

In preliminary experiments by Goldblatt,[1] bilateral adrenalectomy in dogs appeared to interfere with the development or maintenance of the hypertension which follows experimental production of renal ischemia. Similar results were obtained by Blalock and Levy[2] and by Page.[3] On the other hand, Collins and Wood[4] concluded that, "It is unlikely that the adrenal cortex is involved specifically in the etiology of experimental renal hypertension other than in the sense that the cortex is important in the maintenance of blood pressure in normal as well as in hypertensive states."

Our experiments show that this form of experimental hypertension can exist in the complete absence of the adrenal glands, in untreated animals. Seven dogs were subjected to complete bilateral adrenalectomy in addition to constriction of the main renal arteries by means of Goldblatt clamps. Four of these animals were treated

* Aided by the G. N. Stewart Memorial Fund, contributed by The Louis D. Beaumont Trust, N. L. Dauby, Richard H. Kohn, Mrs. Frances W. Lang, and Howard E. Wise.

1 Goldblatt, Harry, *Ann. Int. Med.*, 1937, **11**, 69.

2 Blalock and Levy, *Ann. Surg.*, 1937, **106**, 826.

3 Page, *Am. J. Physiol.*, 1938, **122**, 352.

4 Collins and Wood, *Am. J. Physiol.*, 1938, **123**, 224.

by administration of salt (sodium chloride and citrate, occasionally also bicarbonate) alone or in addition to a commercial extract of adrenal cortex.† In 3 experiments no treatment whatever was employed and the use of salt for seasoning food was avoided. All the blood pressure observations were made by the carotid loop (Van Leersum) method. The cuff was adjusted by the same observer in every case and not less than 12 readings were recorded each time.

Following excision of the second adrenal, in hypertensive dogs, a decided fall of blood pressure sometimes occurs within a day. We have observed a similar fall of pressure in some hypertensive animals after operations other than adrenalectomy. Our experience has shown that return to hypertensive levels of pressure can occur in such adrenalectomized dogs without administration of salt or adrenal extract. The rise in pressure therefore cannot be attributed, with confidence, to treatment when given.

In the 3 untreated adrenalectomized dogs decisive hypertension was maintained in the complete absence of both adrenals. Two of these animals, whose average normal systolic blood pressure was approximately 130 mm and 135 mm Hg, respectively, were subjected to unilateral adrenalectomy and ipsilateral constriction of the renal artery at one operation; later, the same was done on the opposite side. Both dogs succumbed on the fourth day after the second operation, the blood pressure having reached 234 mm and 198 mm, respectively.

The average normal blood pressure in the third of the untreated adrenalectomized dogs ranged between 150 mm and 160 mm. One adrenal was excised and the corresponding renal artery was constricted. Later, the renal artery on the opposite side was constricted. Still later, while marked hypertension was present, the second adrenal was excised. The dog lived upon the ninth day after the complete adrenalectomy. No significant change in the hypertensive level of blood pressure occurred until 2 days prior to the death of the animal. The pressure reached 204 mm Hg on the second day after the adrenalectomy; it was still approximately 200 mm on the eighth day, when the dog was semi-comatose and obviously moribund.

Since the hypertension which follows production of renal ischemia was maintained in untreated, completely adrenalectomized animals, we are led to conclude that neither the cortex nor medulla of the adrenal plays a significant rôle in the etiology of this form of experimental hypertension.

† Generously supplied by The Upjohn Company.

10570

A Simple and Practical Device for the Quantitative Evaluation of Spectral Lines.

Edward C. Hughes. (Introduced by Robert K. Brewer.)

From the Laboratories of Physiology and Physiological Chemistry, Syracuse University, College of Medicine.

The recently developed methods using the quartz spectrograph in the quantitative determination of inorganic elements in biological fluids require some sort of an instrument for the accurate evaluation of the lines on the photographic plate.

Standard densitometers for this purpose are available but their cost is a handicap to laboratories where spectrographic analysis is being conducted on a comparatively small scale. We have tried to overcome this handicap by constructing a simple device from apparatus available in the laboratories.

Inasmuch as only a portion of the photographic deposit is measured it is called a micro densitometer. It is composed of a binocular microscope which serves as the optical system, a microscope lamp with a source of constant current, a sensitive photo electric cell, and a sensitive deflecting galvanometer.

The photo electric cell of the Voltaic type is mounted on one ocular of the microscope while the other ocular is used for vision of the field under examination. In order to measure only the light that is transmitted through the photographic deposit, the high power or 4 mm lens is used and also a metal field restrictor is placed in the ocular below the photronic cell. In the other ocular of the microscope a similar restrictor is placed which differs from its mate only in having a larger opening on each side of the slit so that a considerable portion of the field may be visible for orientation purposes.

A six volt automobile headlight bulb mounted in a microscope lamp and lighted from a storage battery is used for illumination. The microscope lamp is fitted tightly to the microscope and the light is uniformly diffused by placing a ground glass lens between the lamp and the condenser of the microscope.

The plate is mounted on the mechanical stage of the microscope; however, it must be cut in sections in order to fit the stage. For comparison of the results obtainable with this instrument and those obtained in industrial analysis using a standard densitometer, plates from solutions containing aluminum were obtained from an outside source. These plates had been calibrated by using a stepped sector disk and the calibration curve plotted. Molybdenum and tin were

used as internal standards. The working curves were plotted using a known solution of aluminum as the abscissa and the logarithm intensity ratio of the internal standards and the aluminum as the ordinate. The curves obtained by the standard densitometer and our instrument were naturally different owing to the greater deflecting of the galvanometer. However, the results were very comparable and were as shown in Table I.

TABLE I.

	Standard densitometer	"Micro"-densitometer
	Plate A. Aluminum.	
	g	g
Known solution (4)	.0042	.0042
" " (5)	.0082	.0082
Unknown solution	.0068	.0068
	Plate B.	
Known solution (3)	.0042	.0042
" " (4)	.0074	.0074
Unknown solution (1)	.0083	.0082
" " (2)	.0067	.0067
" " (3)	.0067	.0067
	Plate C.	
Known solution (3)	.0042	.0042
" " (4)	.0074	.0074
Unknown A	.0070	.0071
" B	.0066	.0066

10571 P

Effect of Testosterone Propionate on Sex Differentiation in Pouch Young of Opossum.

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The young of the opossum *(Didelphys virginiana)* at birth are sexually almost undifferentiated. Histological differentiation in the gonads has just begun, wolffian ducts are present but müllerian ducts do not appear until several days after birth and are not fully formed until a head length of 9 mm is attained. At about 10 days sex may first be recognized externally by the development of the folds of the marsupium in females and scrotal sacs in males.[1, 2] On

[1] Baxter, J. S., 1935, *Cont. to Embryol.*, No. 145, Carnegie Institution, Washington.

[2] McCrady, Edw., 1938, *American Anatomical Memoirs*, No. 16, Wistar Institute, Philadelphia.

the first or second day after birth injections of testosterone propionate were begun and repeated at intervals of a few days. Prominent changes in the form of the phallus at 10-14 days were the first visible results of treatment.

Three litters received testosterone propionate* for periods of 20, 40, and 50 days in amounts to average 50, 75, and 100 gamma per day. In general, homologous parts in both sexes responded similarly, but not to the same degree. The response was roughly proportional to dosage and duration of treatment. The various parts of the embryonic reproductive system will be taken up in order.

The gonads are affected but little. Testes do not descend (although the gubernacular apparatus is complete) for a reason which will appear. They are somewhat smaller than control testes but appear normal histologically. The interstitial tissue has not yet been studied quantitatively. Ovaries are modified in many ways but are essentially ovaries. Large rete canals, often expanded, are directly connected to the epididymides. Full histological description is impossible within the limits of this report.

Wolffian ducts. Normal vasa deferentia are typically present in experimental animals of both sexes, with well developed epididymides. In older female controls wolffian ducts and epididymides are almost completely degenerate. Occasionally in experimental females small segments of wolffian duct may lack a clear lumen. In younger stages (20 days) the connection of the wolffian duct to the urinogenital sinus via the "sinus cord" is usually non-patent in controls of both sexes and experimental females, but is patent in treated males. In older animals the duct is complete and patent as far as the "sinus cord", which is solid at this stage of development.

Müllerian ducts. The well-known "unorthodox" action of many androgens appears in the case of müllerian duct derivatives, which are enormously enlarged, except for the vaginal region in experimental males. This portion is absent—perhaps because it is first to disappear in normal male development. Uterine and tubal development in males is so great at higher dosages as to prevent descent of the testes.

Urinogenital sinus. This structure is greatly hypertrophied and cornified in both sexes. Prostatic outgrowths are very numerous from the anterior half of the sinus and the adjacent urethra, and are greatly proliferated, especially in males. The bulbo-vestibular and bulbo-urethral glands, arising near the junction of sinus and the urethral groove of the phallus are also proliferated. The "sinus cord", which normally forms the terminal portion of the lateral vagina, is typically enlarged in treated females.

* By the courtesy of Dr. Erwin Schwenk, of the Schering Corporation.

Phallus. This organ responds phenomenally to the androgen in both sexes, but is more reactive in males. Glans, corpus and crural structures are all enlarged, the organ is turgid and the posture erect. The best developed female phalli are as large as the average male, and almost indistinguishable from them.

Scrotum and pouch. These parts, to gross examination, are unmodified. The pouch in females, and the scrotal sacs in males have the same size and appearance as control specimens. The saccus vaginalis is fully formed in males and the gubernaculum properly attached, but testes are undescended, as already noted.

Summary. The administration of testosterone propionate to the pouch young of the opossum induces development of a strange medley of the characters of both sexes. On the male side the phallus, wolffian duct, epididymis, rete, and the glands of the urinogenital sinus are all stimulated, and the effect is commonly greater in males than in females. Müllerian duct derivatives are also stimulated, however, but more so in the female. Differentiation of the gonads is not profoundly affected, and scrotum and pouch are entirely unresponsive. For most structures a sex factor apparently affects the degree of the response.

10572

Demonstration of Streptococcal Fibrinolysin in Exudates. The Action of Sulfanilamide upon It.

Erwin Neter.

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In a preliminary note[1] it was reported that exudates of streptococcal and staphylococcal origin may exhibit fibrinolytic activity. Subsequently, a more detailed study on the occurrence *in vivo* of staphylococcal fibrinolysin was presented.[2] Recently, Tillett[3] described the presence of fibrinolysin in empyema-fluids from which *beta* hemolytic streptococci were recovered. In the following communication, observations are presented dealing with the demonstra-

1 Neter, E., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 735.

2 Neter, E., *J. Bact.*, 1937, **34**, 243.

3 Tillett, W. S., *Bact. Rev.*, 1938, **2**, 161.

tion of fibrinolysin present in various exudates of *beta* hemolytic streptococcal origin and further with the action of sulfanilamide upon the fibrinolysin produced *in vivo* by this microörganism.

For the demonstration of the fibrinolysin present in exudates, the following technic was employed: The exudate was centrifuged and the supernatant fluid in serial dilutions (volume 0.5 cc) was mixed with 1 cc of 1 :5 dilution of human plasma; then 0.25 cc of a 0.25% calcium-chloride solution in normal saline was added; the tubes were shaken thoroughly and incubated at 37°C. The plasma was obtained from healthy human beings by mixing 10 cc of blood with 1 cc of a 2% potassium-oxalate solution; the blood was shaken and centrifuged. Prior to the experiments the plasma was tested as to its susceptibility to the action of streptococcal fibrinolysin. The resulting coagulation of the plasma and dissolution of the clot was noted at various intervals. For the demonstration of the fibrinolysin produced *in vitro,* the supernatant fluid of an 18-hour infusion-broth culture of the respective strain was tested in like manner.

During the last 2 years, 48 specimens of purulent exudates obtained from patients with various infections due to *beta* hemolytic streptococci were tested for fibrinolytic activity. Thirty specimens were found to contain fibrinolysin, while 18 did not. These exudates included 31 empyema-fluids; 15 exhibited fibrinolytic activity, while 16 specimens failed to do so. Four peritoneal exudates were examined and 3 found to contain fibrinolysin. One pericardial exudate was fibrinolytic. Ten specimens of spinal fluid obtained from patients with *beta* hemolytic streptococcal meningitis were tested and 9 were found to exhibit fibrinolytic activity. One arthritic exudate as well as material obtained from a submandibular abscess contained fibrinolysin.

Table I presents the results of an experiment demonstrating the fibrinolytic activity of an arthritic exudate of *beta* hemolytic strep-

TABLE I.
Fibrinolysin in Arthritic Exudate Due to *Beta* Hemolytic Streptococcus.

	Amount of exudate (vol. 0.5 cc) cc	Fibrinolysis After addition of plasma and calcium-chloride solution for		
		20 min	3 hr	8 hr
1.	.5	Negative	Negative	Negative
2.	.05	,,	,,	Positive
3.	.005	,,	Positive	,,
4.	.0005	,,	Negative	Negative
5.		,,	,,	,,

Negative = No fibrinolysis (clot-formation).
Positive = Complete fibrinolysis.

tococcal origin. It is interesting to note that the undiluted exudate failed to exhibit fibrinolytic activity, while the exudate in dilutions of 1:10 and 1:100 dissolved the clot. This observation was repeatedly made on specimens of spinal fluid, empyema-fluid, and peritoneal exudate. On the other hand, other specimens showed strongest fibrinolytic activity when used undiluted. In the experiment presented in Table I, fibrinolysis occurred after 3 hours' incubation at 37°C. In tests with other exudates, fibrinolysis was observed after only one hour of incubation. This finding indicates that fibrinolysin was present in the exudate and was at least not entirely formed during possible growth of the hemolytic streptococcus in the exudate-plasma mixture. Experiments with Seitz and Berkefeld filtrates of exudates failed to yield uniform results. In 2 instances only, did the sterile filtrates exhibit fibrinolytic properties.

During the last 2 years, 5 patients with empyema due to *beta* hemolytic streptococcus were treated in this hospital with sulfanilamide and prontosil, respectively. Of these patients, 4 recovered and one died. The fibrinolytic activity of the exudates obtained at various periods prior to, and during the treatment of the patients with sulfanilamide was studied. The findings in one of these cases were as follows: On 3 consecutive days the empyema-fluid exhibited fibrinolytic activity (titer, 1:100), while subsequently fibrinolysin could no longer be demonstrated. Mention may be made that during the 9 days of observation, the cultures of the empyema-fluid revealed the presence of *beta* hemolytic streptococci. It is interesting to note that following the administration of sulfanilamide, the specimen of empyema-fluid taken on the third day of observation exhibited fibrinolytic activity in spite of the presence of 5.5 mg % of sulfanilamide in the fluid. Specimens taken later during the illness, however, lacked fibrinolytic activity; the sulfanilamide concentration in these specimens ranged from 6.7 mg % to 10.9 mg %. Whether or not the lack of fibrinolytic properties of these specimens of empyema-fluid is related to the continued administration of sulfanilamide cannot be decided at the present time. Another possible mechanism responsible for the disappearance of demonstrable fibrinolysin in exudates is the production of antifibrinolysin. Tillett[3] observed in 2 instances that at a time when the empyema-fluid, which previously had shown fibrinolytic activity, became thick with fibrin, antifibrinolytic properties were demonstrable in the blood of his patients. In the case presented above, however, the plasma (as well as the serum) of the patient lacked antifibrinolytic properties at a period when fibrinolysin was not demonstrable in the empyema fluid any longer.

It may be added in this connection that the strain of hemolytic streptococcus isolated from the empyema-fluid during the entire period of observation remained strongly fibrinolytic *in vitro*, although it had been exposed *in vivo* to sulfanilamide in concentrations of 5.5 to 10.9 mg % for 5 days.

It was observed by several authors, including Madison and Snow,[4] Huntington,[5] and Kemp,[6] that sulfanilamide failed to neutralize fibrinolysin formed *in vitro* by *beta* hemolytic streptococci. The possibility may be considered that sulfanilamide may counteract fibrinolysin that was produced *in vivo*.[7] To this end, the action of sulfanilamide upon fibrinolysin present in peritoneal exudates of mice infected with *beta* hemolytic streptococci was tested. The experiment was carried out in the following way: Decreasing amounts (volume 0.5 cc) of 0.8% sulfanilamide dissolved in physiological saline solution were mixed with 0.5 cc of a 1:10 diluted peritoneal exudate and incubated for 2 hours; then 0.2 cc of plasma, 0.3 cc of saline, and 0.25 cc of the calcium-chloride solution were added. The experiments revealed that sulfanilamide failed to retard or inhibit the fibrinolytic activity of the peritoneal exudate.

10573

Non-Effect of a High Yeast Diet on Survival of Adrenalectomized Rats.

ROSE MARRAZZI AND ROBERT GAUNT.

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Verzar[1-5] and his associates have developed a theory that the function of the adrenal cortical hormone is to maintain adequate phosphorylation processes in metabolism. One manifestation of this

4 Madison, R. R., and Snow, J. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 592.

5 Huntington, R. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 328.

6 Kemp, H. A., *Texas State J. Med.*, 1938, **34**, 208.

7 Neter, E., *Arch. Path.*, 1938, **26**, 1082.

1 Laszt, L., and Verzar, F., *Pflüger's Arch.*, 1935, **236**, 693.

2 Verzar, F., and Laszt, L., *Pflüger's Arch.*, 1936, **237**, 476.

3 Laszt, L., and Verzar, F., *Pflüger's Arch.*, 1937-8, **239**, 136.

4 Laszt, L., and Verzar, F., *Pflüger's Arch.*, 1937-8, **239**, 653.

5 Verzar, F., and McDougall, E. J., *Absorption from the Intestine*, Longmans, Green & Co., London.

TABLE I.

No. of rats	Treatment	Avg days survival	Range, days	No. living indefinitely
59	Yeast	7.3	4-17	4
56	Untreated	8.1	4-16	5

action is the transformation of dietary flavin (pro-vitamin G) to flavin phosphate (vitamin G), a process which, according to them, can not go on in the absence of cortical hormone. A crucial point in their argument is the claim that flavin phosphate, as present in liver extracts or in yeast[1, 2, 3] will extend the lives of adrenalectomized animals (rats), whereas non-phosphorylated flavin will not. This report receives support from the experiments of Pijoan and Oberg,[6] and is compatible with the results of Sandberg and Perla,[7] who found that a special diet, one item of which was 5% yeast, helped maintain their adrenalectomized rats.

We report here non-confirmatory studies concerning the effects of a high yeast diet on the survival of adrenalectomized rats.

A total of 115 rats of both sexes, approximately 30 days of age at the time of operation, were used. Litters were divided and half were adrenalectomized and yeast-treated; half were kept as untreated adrenalectomized controls. Treatment was as follows:

28 received brewer's yeast† to the extent of 5% of the stock diet,* starting 5 to 7 days before adrenalectomy.

25 received brewer's yeast to the extent of 10% of the diet, starting 2 to 10 days before adrenalectomy.

6 received baker's yeast‡ to the extent of 10% of the diet, starting 6 days before adrenalectomy.

During the preadrenalectomy observation period any animals not gaining weight normally were discarded.

The results are shown in Table I. Since no difference was found in the various groups receiving different types and amounts of yeast, all results are pooled. No suggestion of a life extension as a result of yeast treatment was found; and in other respects such as weight

6 Pijoan, M., and Oberg, S. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 187.

7 Sandberg, M., and Perla, D., *J. Biol. Chem.*, 1936, **113**, 35.

* The stock diet used is made as follows: GLF Calf Meal, 88.8%; ground meat scraps, 8.9%; brewer's yeast, 0.7%; cod liver oil, 1.65%.

† Each gram contained not less than 25 international units of vitamin B_1, and not less than 42 Sherman units of B_2 (G). This was kindly supplied through the courtesy of Mead Johnson & Co.

‡ Each gram contained not less than 20 Sherman units of B_2 (G). This was kindly supplied through the courtesy of Mr. Charles N. Frey of the Fleischmann Laboratories.

changes, etc., treated and control animals responded in an identical fashion.

Of the animals that survived indefinitely, presumably because of accessory cortical tissue, those in the treated group continued to gain weight just as well when the yeast supplement of the diet was discontinued.

Summary. Fifty-nine adrenalectomized rats were fed a high yeast diet and their survival compared to 56 control animals on a stock diet. Reports that there is a factor in yeast (presumably vitamin G) which will extend the lives of adrenalectomized rats were not confirmed. The survival of treated animals was entirely within normal range.

10574 P

Conversion of 1,2,5,6-Dibenzanthracene by Rabbits, Rats, and Mice. Significance in Carcinogenesis of this Conversion.

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Boyland studied chemically the conversion of certain carcinogenic as well as non-carcinogenic compounds.[1, 2] This communication reports a study, by combined chemical and physical methods, of the conversion and excretion of 1,2,5,6-dibenzanthracene by rabbits, rats, and mice. Ether extracts of the urine and feces of injected animals were examined by spectroscopy in the ultraviolet region of the spectrum with the hydrogen discharge tube as a source of light.

Rabbits, rats, and mice were injected subcutaneously or intramuscularly with 2-10 cc of a solution containing 500 mg of pure 1,2,5,6-dibenzanthracene in 100 cc of olive oil. The urine and feces of the injected animals were collected from metabolism cages at intervals of 3 days.

The urines were acidified with HCl and extracted continuously with ether for 48-72 hours. The ether was extracted (1) with 10% Na_2CO_3 solution to separate the acid compounds, and then (2) with a 10% NaOH solution to separate the phenolic compounds. The residual ether contained the neutral compounds. The extracts (1) and (2) were next acidified with HCl, and reëxtracted with ether.

1 Boyland, E., and Levi, A. A., *Biochem. J.*, 1935, **29**, 2679; 1936, **30**, 728, 1225.

2 Levi, A. A., and Boyland, E., *J. Soc. Chem. Industry*, 1937, **61**; *Chem. and Industry*, **15**, 446.

The 3 ether solutions were concentrated to a volume of 25 cc each and examined spectroscopically.

The feces were acidified with HCl, mixed with anhydrous Na_2SO_4, ground to a powder, and extracted as were the urines.

1. *Fraction containing ether-soluble, acid substances.* Feces and Urine: This fraction from injected rabbits, rats, and mice gives no absorption which suggests the presence of acid conversion products of dibenzanthracene.

2. *Fraction containing ether-soluble, phenolic substances.* Feces and Urine; Rabbits: This fraction from rabbits injected with dibenzanthracene gives a group of absorption bands not given by similar fractions from the excreta of normal rabbits, and not in the positions of the bands given by dibenzanthracene.

Feces and Urine; Rats and Mice: This fraction from injected rats and from mice gives identical absorption bands. They do not resemble those of dibenzanthracene and are not given by a similar fraction from normal rats and mice. Traces of similar bands are given by the urine of injected rats and mice.

The positions of the absorption bands in this fraction from injected rats and mice differ from those of the bands given by a similar fraction from injected rabbits. This suggests that dibenzanthracene is metabolized differently in different species. The presence of bands in a fraction designed to contain phenolic compounds suggests the presence of a phenolic derivative of dibenzanthracene.

3. *Fraction containing ether-soluble, neutral substances.* Feces: Absorption bands are given by this fraction from injected rats, mice, and *rarely* from rabbits. Their positions are similar to those of the bands given by an ethereal solution of dibenzanthracene. No bands suggest the presence of any neutral conversion product of dibenzanthracene.

Urine: No bands are given by this fraction from injected rabbits in positions similar to those of dibenzanthracene. No indication of the presence of any neutral conversion product of dibenzanthracene has been found. A similar extract from injected rats and mice occasionally gives the absorption bands of dibenzanthracene, but this finding is probably an artefact.

To isolate the phenolic conversion product of dibenzanthracene a large number of rabbits and rats were injected weekly with that compound. The ether-soluble phenolic material was isolated from the excreta, freed from contaminants by high vacuum distillation, and further purified. From the rabbits about 70 mg of a crystalline material* and from the rats about 5 mg of a uniform, fairly pure

* Dr. Boyland kindly supplied an absorption curve of dibenzanthracene deriva-

material was obtained. This crystalline material obtained from rabbits has proved to be non-carcinogenic for 9 mice that have been under observation 6 months after injection. Nine litter-mate controls injected with the same amount of dibenzanthracene have developed tumors. This observation suggests that in certain animals an immunity to carcinogenesis by chemicals depends upon the ability to convert the carcinogen from a neutral to a phenolic compound.

Summary. From the extracts of excreta of rabbits, rats, and mice injected with 1,2,5,6-dibenzanthracene, substances of a phenolic nature were isolated which are considered to be conversion products of dibenzanthracene. The substance isolated from injected rabbits was non-carcinogenic and gave absorption bands different from those of the substance obtained from injected rats and mice. This fact suggests that different species metabolize dibenzanthracene differently. The absorption bands of unchanged dibenzanthracene were present in the fraction containing neutral compounds of feces of injected rats, mice, and *rarely* of rabbits.

10575 P

A "Sulfapyridine-Fast" Strain of Pneumococcus Type I.

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Sulfapyridine exerts *in vitro* a bacteriostatic effect on the pneumococcus under aërobic conditions. It was of interest to determine whether a strain of this organism could be adapted to growth in increasing concentrations of the drug and so become "sulfapyridine-fast." The acquisition of "sulfapyridine-fastness" by a strain of pneumococcus has been reported recently by Maclean, Rogers, and Fleming.[1]

A mouse-virulent strain of pneumococcus Type I (SV-I) was used in the present experiments. The stock solutions of sulfapyridine were made by dissolving the drug in N/10 HCl, diluting with distilled water and neutralizing with N/10 NaOH. The dilutions of sulfapyridine in 2% serum-broth were stored in the ice-box.

tive isolated from rabbits' urine. The shape of the curve and the positions of the maxima and minima are identical with those of the absorption curve of the phenolic derivative isolated by us from rabbits.

[1] Maclean, Rogers, and Fleming, *Lancet*, 1939, **1**, 562.

The strain of pneumococcus was transferred serially in serum-broth containing sulfapyridine, beginning with a concentration of 1:160,000 and increasing gradually to a concentration of 1:16,000. By this process the pneumococcus became adapted to growth in relatively high concentrations of sulfapyridine.

Throughout the period of adaptation no alteration in the morphology of the organism was apparent; the colonies on blood agar were smooth, and no change in its specific immunological characteristics was demonstrated.

After 33 transfers in serum-broth containing sulfapyridine, the strain which had become tolerant to high concentrations of the drug *in vitro,* was tested in experimental infections of mice to determine whether "sulfapyridine-fastness" could be demonstrated *in vivo* as well.

In Table I are shown the results of an experiment in which mice were infected intraäbdominally with the "drug-fast" strain and treated with sulfapyridine, as compared with the results observed when the parent strain of pneumococcus was used under similar conditions.

TABLE I.

Results of Treatment with Sulfapyridine of Mice Infected with Parent Strain and "Sulfapyridine-fast" Strain of Pneumococcus Type I.*

Strain of pneumococcus Type I	Infecting dose cc of culture	Treatment with sulfapyridine	Result
SV-I	10–2	3 doses, 30 mg, 2 days	S S D†
Parent strain			S S D
			S S D
	10–2	4 " 30 " 3 "	S S S
			S S S
			S S S
	10–7	0	D
			D
	10–8	0	D
			D
SV-I/P/43	10–2	3 " 30 " 2 "	D D D
"Sulfapyridine-fast" strain			D D D
			D D D
	10–2	4 " 30 " 3 "	D D D
			D D D
			D D S
	10–7	0	D
			D
	10–8	0	D
			S

*All mice were injected intraäbdominally. The drug was administered by stomach-tube in 30 mg doses. The first dose was given immediately following infection, the second five hours later. Subsequent doses of 30 mg each were given at daily intervals.

†D indicates death; S indicates survival.

Six out of 9 mice infected with the parent strain (SV-I) survived when treated with sulfapyridine for 2 days, a total of 90 mg being given. However, all of the mice died which were infected with the "sulfapyridine-fast" strain, (SV-I/P/43) and treated with sulfapyridine for 2 days. In other groups of mice sulfapyridine was given over a period of 3 days, the total amount of drug administered being 120 mg. All of the treated mice which were infected with the parent strain survived, whereas all but one of the mice died after infection with the "sulfapyridine-fast" strain and similar treatment with sulfapyridine.

The "sulfapyridine-fast" strain retains the typical lanceolate form and is gram-positive. There is no evidence of dissociation to the rough phase and its virulence for mice is unimpaired. Likewise it retains its specific immunological characteristics. It shows the typical "quellung" phenomenon in Type I antipneumococcal rabbit serum and is agglutinated specifically by Type I antiserum. Mice infected with the "drug-fast" strain are protected by Type I antiserum, and mice immunized with the parent strain are resistant to infection with the drug-fast strain.

The acquisition of "sulfapyridine-fastness" by pneumococcus Type I under these circumstances appears to be relatively permanent. After 30 serial transfers in broth not containing the drug, sulfapyridine-fastness was retained. Similarly fastness was still present after 10 passages in untreated mice.

Summary. A strain of pneumococcus Type I has been made "sulfapyridine-fast" by repeated transfers in broth containing increasing concentrations of sulfapyridine. "Sulfapyridine-fastness" is demonstrable both *in vitro* and *in vivo*. The alteration is not associated with changes in morphology, virulence, or specific immunological characteristics.

10576

Effect of Sulphur-Amino Acid Deficiency on Wound Healing.*

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From the results of his numerous plant and animal experiments, Hammett[1, 2, 3] arrived at the conclusion that the sulphydryl radicle, naturally present in all cells, was the essential and universal stimulus to growth by increase in the number of cells. This hypothesis did not long remain unchallenged. Others,[4, 5] after carrying out similar investigations, have either not been able to reproduce Hammett's results, or have preferred to lend to them another interpretation. Reimann[6, 7, 8] translated Hammett's work into the clinic and reported instances of chronic indolent ulcers which healed with dramatic swiftness following the direct application of sulphydryl compounds. He described a rapid proliferation of both the fibroblastic and epithelial elements of the wound.

In recent years Rose[9, 10] has demonstrated methionine and not cystine to be the indispensable sulphur-containing amino-acid. Cystine stimulated growth only in the presence of methionine. In the absence of the latter from the ration, however, the animals rapidly lost weight and died, even though an abundant amount of cystine had been supplied.

The purpose of these experiments was to determine whether a deprivation of cystine and methionine in a degree sufficient to abruptly arrest growth, had any effect on the healing of soft tissue wounds. The tensile strength of the wound was used as an index of healing. Young growing rats weighing between 90 and 100 g were divided

* Aided by a grant from the Fluid Research Funds of the Yale University School of Medicine.

1 Hammett, F. S., *Protoplasma*, 1929, **7**, 297.

2 Hammett, F. S., and Reimann, S. P., *J. Exp. Med.*, 1929, **50**, 445.

3 Hammett, F. S., *Protoplasma*, 1931, **13**, 331.

4 Hueper, W. C., *Arch. Path.*, 1934, **17**, 218.

5 Hueper, W. C., Allen, A., Russel, M., Woodward, G., and Platt, M., *Am. J. Cancer*, 1933, **17**, 74.

6 Reimann, S. P., and Hammett, F. S., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 20.

7 Reimann, S. P., *J. A. M. A.*, 1930, **94**, 1369.

8 Reimann, S. P., *Ann. Surg.*, 1931, **93**, 624.

9 Rose, W. C., *Science*, 1937, **86**, 298.

10 Rose, W. C., *Physiol. Rev.*, 1938, **18**, 109.

into 2 groups: (1) normal control, (2) sulphur-amino-acid deficient. The experimental diet used was that described by Dyer and du Vigneaud,[11] and consisted of:

Casein	6.0
Dextrin	37.0
Sucrose	15.0
Lard	19.0
Cod liver oil	5.0
Salt mixture	4.0
Agar	2.0
Milk vitamin concentrate	12.0

This is a full diet, adequate in all the essential constituents except the sulphur-bearing amino-acids. It contains cystine and methionine sufficient only for the maintenance of the animal but not for normal growth and increase in weight.

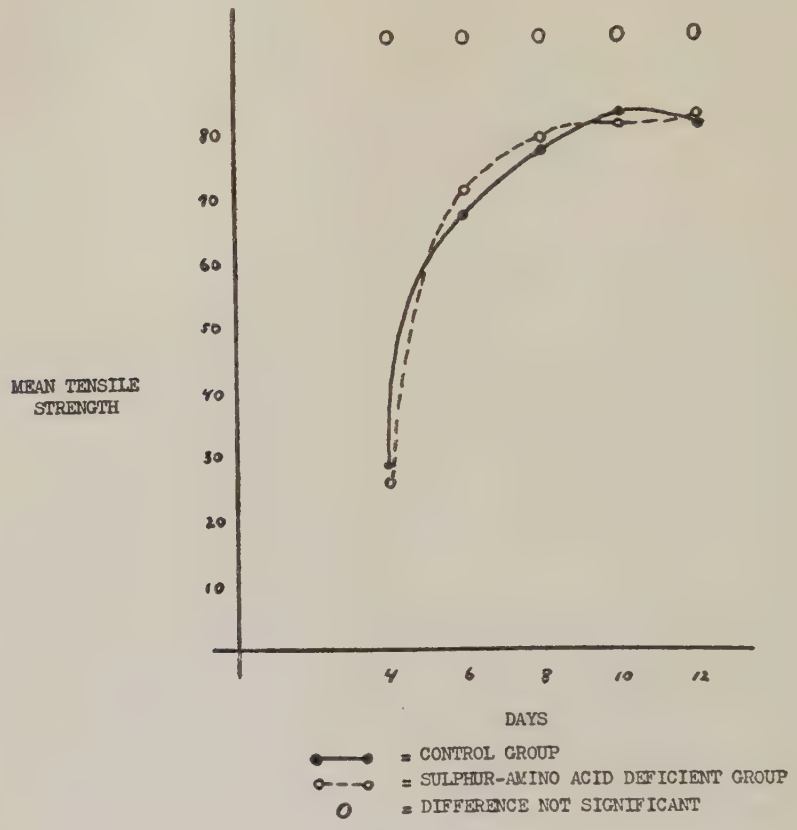
I. Control group—20 animals. This group was maintained throughout the duration of the studies on the experimental diet supplemented with 0.6 g of crystalline cystine. The animals continued to grow and gained an average of one gram in weight per day. Those which did not gain weight were discarded. On the 10th day a longitudinal wound measuring about one cm in length was made under ether anesthesia through the anterior wall of the stomach at its cardiac end. The wound edges were immediately reapproximated in one layer with a running continuous Connell suture of No. 000 plain catgut which, as has been previously shown, loses its tensile strength well within the fourth day.[12] The abdominal wall was closed with 2 layers of fine No. A silk. Strict aseptic precautions were observed. On each of the 4th, 6th, 8th, 10th, and 12th postoperative days 4 animals were sacrificed, and the strength of the wound immediately determined by distending the stomach with air and noting its bursting point. The details of this method were described by Harvey and Howes.[13] Inasmuch as it has been shown that for approximately 4 days after the injury, the wounds had only the strength contributed by the holding power of the sutures, no studies were made during this first phase of healing.

II. Sulphur-amino-acid deficient group—30 animals. This group was maintained on the experimental diet alone. All the animals abruptly ceased to grow. Some slowly lost weight but all survived throughout the duration of the experiment. Although pure amino-acid mixtures were not used, the survival of the animals in this

11 Dyer, H. M., and du Vigneaud, *J. Biol. Chem.*, 1935, **109**, 477.

12 Howes, E. L., *J. A. M. A.*, 1928, **90**, 530.

13 Harvey, S. C., and Howes, E. L., *Ann Surg.*, 1930, **91**, 641.



GRAPH I.

group and their failure to grow indicate, in an otherwise complete ration, a deficiency of methionine as well as cystine. On the 10th day, wounds exactly similar to those of the control group were made. Determinations of the tensile strength were carried out from the 4th through the 12th post-operative day.

In one of the members of the control group a small walled off abscess was found adjacent to the operative site in the stomach. This animal was discarded. In the remaining 49 animals of both series, the stomach and abdominal wounds all healed *per primam*. No troublesome adhesions were encountered. In each group the averages of the breaking strength for every postoperative interval, as well as the standard deviation, were computed. Fisher's[14] formula for small samples was applied to determine whether the difference in the means of the two groups was statistically significant.

[14] Fisher, R. A., *Statistical Methods for Research Workers*, Edinburgh, 1934.

Results. No significant deviation from the normal healing of stomach wounds was discovered in young rats maintained upon a ration deficient in cystine and methionine. The curves of healing of these wounds, as measured by their tensile strengths, were practically identical in the control and in the experimental groups. (Graph I.) (The distended stomachs ruptured at places elsewhere than the operative sites in 10 of the 11 control animals, and in 15 of the 17 experimental animals for the 8-, 10- and 12-day periods.) These findings do not warrant any opinion concerning the allegedly essential rôle of sulphydryl compounds as the real stimuli to cell growth and proliferation. Nor can any conclusions be justly drawn regarding the absolute indispensability of the sulphur-bearing amino-acids in the process of fibroblastic proliferation and wound healing. If these substances are indeed essential in this process, then it must be postulated that the animal, not provided with enough of the sulphur compounds in its diet to allow adequate body growth, is still able to derive from some other source a quantity sufficient to permit adequate wound healing. This source is possibly endogenous and may be related to the breakdown of its own body proteins.

10577

Biological and Immunological Identity of Toxoplasma of Animal and Human Origin.

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There exists considerable confusion among parasitologists as to the characteristics which justify the classification of Protozoa of certain morphology as *Toxoplasma.* The capacity to multiply and to produce disease in a variety of hosts, including mammals and birds, must, in accord with the conclusions of Aragao,[1] be regarded as the chief taxonomic characteristics of the group. Morphology as the only guide can be misleading and confusing as is evident from the present controversy as to whether certain forms observed in avian malaria are *Toxoplasma* or stages of *Plasmodium.*[2] In accord with Aragao's criteria, the existence of *Toxoplasma* in North America was first demonstrated by Sabin and Olitsky,[3] who isolated *Toxo-*

1 Aragao, H. de B., *Compt. rend. Soc. biol.*, 1933, **113**, 214.

2 Hegner, R., and Wolfson, F., *Am. J. Hyg.*, 1938, **27**, 212; *ibid.*, 1938, **28**, 437.

3 Sabin, A. B., and Olitsky, P. K., *Science*, 1937, **85**, 336.

plasma from the brain of a guinea pig and showed them to be pathogenic for guinea pigs, rabbits, mice, monkeys and chickens. The forms seen by Mooser[4] in guinea pigs in Mexico and by Markham[5] in the United States may have been *Toxoplasma* but in the absence of transmission-experiments, the diagnosis remained uncertain. The reports by Manwell and Herman,[6] Herman,[7] and Wood and Wood[8] of the presence of *Toxoplasma* in North American birds cannot be accepted as proved, since the identification was made only on morphologic grounds, while transmission to other birds was unsuccessful and no tests were made on mammals. The parasites identified by Wolfson[9] as *Toxoplasma* in canaries were transmitted to other canaries but no tests with mammals were reported. Similarly it may be said that while human infection with *Toxoplasma* has been suggested by several investigators on morphological grounds, it has not hitherto been proved by adequate animal transmission and identification. The circumstances under which Bland[10] obtained *Toxoplasma* in a rabbit after inoculation with blood from a patient suffering from glandular fever (infectious mononucleosis) were such that one could not be certain whether they originated in the human blood or the rabbit. In view of the demonstration that monkeys recovering from experimental toxoplasmosis developed neutralizing antibodies for the parasites,[3] a number of sera from patients recovered from infectious mononucleosis were tested for such antibodies against our *Toxoplasma* and none was found.

In my opinion the first definite evidence that *Toxoplasma* can infect human beings has just been supplied by Wolf, Cowen, and Paige[11] with a case of encephalitis in a child. They not only demonstrated parasites of typical morphology in the human tissues but isolated *Toxoplasma* from a large number of rabbits and mice that were injected with the human brain. The fact that they used a large number of animals for transmission and that so many of them developed the infection almost simultaneously after a suitable incubation period leaves little doubt that the parasites originated in the human tissue. After several passages in mice these investigators submitted to this laboratory for a comparative study their *Toxo-*

4 Mooser, H., *J. Infect. Dis.*, 1929, **44**, 186.

5 Markham, F. S., *Am. J. Hyg.*, 1937, **26**, 193.

6 Manwell, R. D., and Herman, C. M., *J. Parasitol.*, 1935, **21**, 415.

7 Herman, C. M., *Am. J. Hyg.*, 1937, **25**, 303; *Tr. Am. Micr. Soc.*, 1938, **57**, 132.

8 Wood, F. D., and Wood, S. F., *J. Parasitol.*, 1937, **23**, 197.

9 Wolfson, F., *J. Parasitol.*, 1937, **23**, 553.

10 Bland, J. O. W., *Lancet*, 1930, **2**, 521; *Brit. J. Exp. Path.*, 1931, **12**, 311.

11 Wolf, A., Cowen, D., and Paige, B., *Science*, 1939, **89**, 226.

plasma of human origin in the form of an infected mouse. As regards pathogenicity for a wide host-range, including mammals and birds, the *Toxoplasma* of human (Hum.) origin corresponded in every respect to those of animal (An.) origin. Mice injected intracerebrally (0.03 cc) and intraäbdominally (0.5 cc) with infected mouse-brain died with nervous signs after incubation periods of 5 to 8 days. When the inoculation was made only intraäbdominally, all mice became sick, many with nervous signs, and the majority died while some survived with chronic disease and infection. The parasites seen in films of the peritoneal exudate, the brain, and viscera were morphologically identical with the ones studied in this laboratory for the past 4 years. Intracutaneous injection of 0.2 cc to 0.3 cc of infected mouse-brain suspension on the back of rabbits, was followed by the development (after 3 to 4 days) of a characteristic indurated skin-lesion, the center of which eventually underwent hemorrhagic necrosis, and of a cycle of fever of 5 to 8 days' duration, terminated by either death or recovery; of four rabbits inoculated in this manner, 2 died and 2 recovered. Not only were the cutaneous lesions and the clinical course indistinguishable from those induced by the "An." *Toxoplasma,* but pathologically there was also a striking similarity in the presence of necrotic foci in the viscera, especially the liver.

Two 1-day-old chicks (Rhode Island Reds) inoculated intracerebrally with 0.06 cc of a 10% suspension of infected mouse-brain developed nervous signs on the 5th and 6th days respectively. One died on the 6th day and the other was sacrificed when *in extremis. Toxoplasma* were demonstrated in stained impression-films of their brains and by passage to other chicks. Of two 3-weeks-old chicks inoculated intracerebrally with 0.1 cc of the same mouse-brain suspension, one exhibited transitory weakness and very slight incoordination on the 5th and 6th days, while the other showed no signs of disease; at the end of 4 weeks *Toxoplasma* were demonstrated in the brains of both these chicks by combined intracerebral and intraäbdominal inoculation of mice. Passage to other chicks was possible when the infected chick brain was injected intracerebrally but not intramuscularly (Table I).

The immunological identity of the *Toxoplasma* of animal and human origin was established by active cross-immunity and by neutralization-tests. Two rabbits that had recovered from an intracutaneous inoculation of "An." *Toxoplasma* were injected intracutaneously with "Hum." *Toxoplasma* along with 2 normal controls. The 2 convalescents remained well without developing either the typical skin-lesion or fever, while the controls contracted the charac-

TABLE I.
Pathogenicity of *Toxoplasma* of Human Origin for Chicks.

Experiment	Source of *Toxoplasma*	Route of inoculation	Dose, cc	Age of chicks	Chick No.	Result	Remarks
A	Mouse-brain suspension*	Intracerebral	.06	1 day	1	CNS 5th, dead 6th	*Toxoplasma* present in film of brain
					2	CNS 6th, sacrificed 6th	" " " " " " Brain-passaged to other chicks
			.10	3 wk	3	Slight CNS 5th, 6th; recovered	*Toxoplasma* in brain 4 weeks after inoculation demonstrated by mouse-passage
					4	No signs of illness	*Toxoplasma* in brain 4 weeks after inoculation demonstrated by mouse-passage
B	Chick-brain suspension	Intracerebral	.06	5 days	5	CNS 6th, dead 7th	*Toxoplasma* present in film of brain
					6	Slight CNS 7th, 8th; recovered	*Toxoplasma* in brain 4 weeks after inoculation demonstrated by mouse-passage
		Intramuscular	1.00	5 "	7	No signs of illness	Brains, lungs, and spleens injected in mice 1 month after inoculation; no *Toxoplasma* obtained
					8	" " " "	
					9	" " " "	

*Four mice inoculated intracerebrally with 0.03 cc of same suspension exhibited signs of encephalitis on 5th day and were dead on the 6th.

CNS 5th = Signs of encephalitis on 5th day.

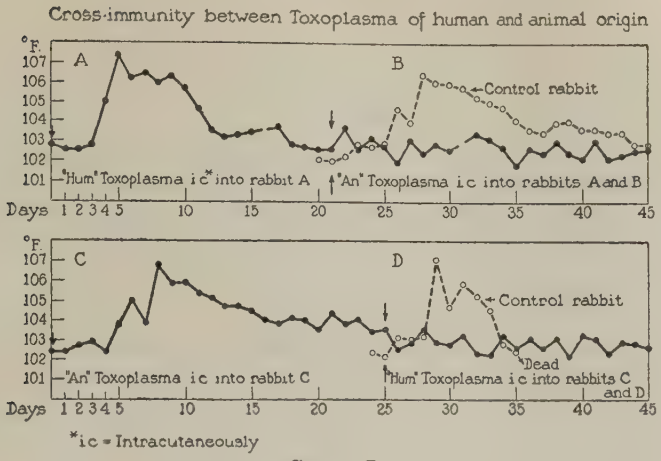
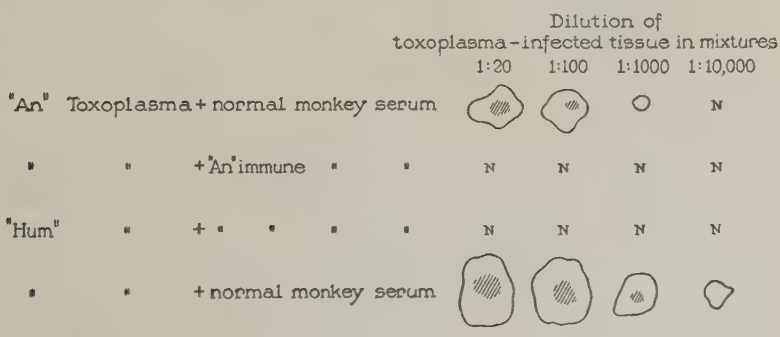


CHART I.

teristic disease. The same result was obtained when 2 "Hum." *Toxoplasma* convalescents were reinoculated with "An." *Toxoplasma* along with 2 controls. Chart 1 shows the temperatures of one set of rabbits in each test. The neutralization-test was carried out with a hyperimmune monkey's serum prepared against the "An." *Toxoplasma* in 1935. A 10% suspension in Tyrode's solution of infected mouse-brain suspension was allowed to sediment spontaneously for a half hour; the supernate and dilutions prepared from it in Tyrode's solution were mixed with equal amounts of undiluted immune or normal monkey's serum. After 10 minutes at room-temperature

Neutralization of Toxoplasma of human and animal origin by serum of monkeys hyperimmunized with "Animal" Toxoplasma



Mixtures injected intracutaneously on the back of a single rabbit. Resulting lesions traced on 12th day

////// = Necrosis N = No lesion

CHART 2.

0.2 cc of each mixture was injected intracutaneously on the back of a single rabbit. The results are shown in Chart 2.

The remarkable immunological and biological identity between the *Toxoplasma* of animal origin and the first strain of human origin suggests that the same protozoön may operate in all susceptible mammals, a fact which must be considered in the epidemiology of toxoplasmosis. The incidence of toxoplasmosis in animals and human beings remains to be determined, and the existence of clinically inapparent or unrecognized non-fatal cases will very likely be found to play a definite rôle in the dissemination of the infection. These studies also suggest that unless the parasites of birds which resemble *Toxoplasma* morphologically, but are not pathogenic for or do not multiply in mammals, can be shown to possess some immunological relationship to the classical *Toxoplasma,* they should be included in a separate group.

Conclusions. *Toxoplasma* of animal and human origin have been shown to be identical biologically in their pathogenicity for mammals and birds, and immunologically by producing an active immunity against one another and by the fact that a serum against one neutralizes both.

10578 P

Hypercalcification, -Calcemia and -Lipemia in Chickens Following Administration of Estrogens.

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Previous studies have shown that pigeons treated with estrogenic substances show a decided rise in blood calcium and a replacement of the marrow cavity by newly-formed endosteal bone.[1] It seemed of interest, therefore, to study the effect of estrogen on the bones and on the blood calcium of the domestic fowl. While our experiments were in progress, Zondek reported that estrogens increase bone calcification

* These investigations were supported in part by the Research Funds of Yale University School of Medicine.

† Further support has been extended by the Anna Fuller Fund and the Jane Coffin Childs Memorial Fund.

1 Pfeiffer, C. A., and Gardner, W. U., *Endocrinology,* 1938, **23**, 485.

and raise the blood calcium level of chicks of both sexes.[2] These changes were associated with dwarfism.

Our material consisted of fully-grown and sexually-mature cocks. We used normal and Creeper cocks, the latter belonging to a breed characterized by chondrodystrophy-like disproportionate dwarfism. Except for one cock, which was 9 months of age, the animals were about 6 to 7 months old when the injections were begun. The body weight of the animals ranged from 870 g to 1620 g at the beginning of the experiment. Nine Creeper and 6 normal cocks were treated with daily injections of estradiol benzoate‡; 5 Creeper and 5 normal cocks served for controls. In addition, similar tests were made with 2 capons (one normal, one Creeper), and 2 capons were used for control. The hormone injections were continued for various periods, as little as 3 weeks in some cocks, and up to over 4 months in others. The daily dose of estrogen varied between 1,700 and 30,000 I. U. With one exception, the cocks made normal gains in body weight during the experimental period.

At the beginning of the experiment the cocks still had rather small combs and wattles. During the course of the injections they decreased in size and assumed the typical capon-like appearance. The cock which was 9 months old when the injections were started, though he responded to the treatment in very typical fashion, still had a large, though somewhat mealy-looking comb after 3 weeks when the experiment was concluded. The testes of the treated cocks were very small, with one exception weighing less than one gram. The testes of the 9-months-old cock weighed 11 g when he was killed at the age of 305 days (about half the normal weight). Six of the 15 treated cocks showed some development of Müllerian rudiments.

After 3 weeks of daily treatment with approximately 1,700 I. U. of estradiol benzoate, inspection of the femur and tibia showed a thin layer of newly deposited calcium on the endosteal surface of the bones. With continued treatment the bones showed more extensive ossification. Treatment with 10,000 I. U. of the hormone daily produced within 3 weeks nearly as extreme bone changes as those found after a much longer period of treatment with smaller amounts of hormone. Deposition of new bone occurred uniformly throughout the epiphyses and diaphyses. The walls were uniformly thickened and trabeculae of spongy bone invaded the marrow. In the most extreme cases the marrow spaces of the metaphyseal regions were

2 Zondek, B., *Folia Clin. orient.*, 1937, **1**, 1.

‡ The estradiol benzoate was generously supplied by Schering Corporation through the courtesy of Dr. E. Schwenk.

nearly obliterated and the marrow cavity was much narrowed near the center of the diaphysis. A definite relationship was found between the daily dose of injected hormone and the extent of new calcification.

The serum calcium of our control cocks varied from 8.96 to 9.61 mg %.§ With one exception, all cocks which received daily injections of 4,600 I. U. or more of estrogen showed increased serum calcium values (11.04 to 78.00 mg %). In 4 cases these values were in excess of 40 mg %. The one exception relates to a cock who had lost weight before he was killed and who was in very poor condition. The extent of the bone changes was related in part to the serum calcium level.

The cocks which had received the highest daily amounts of estrogens had extreme lipemia.[3] The blood serum was a deep canary yellow color, very turbid, and on standing quite a large amount of fat would rise to the surface. The highest figures for lipids were: fatty acids 573.2 m eq., cholesterol 1059.0 mg %, free cholesterol 790.0 mg %, lipid phosphorus 201 mg %, compared to the highest value found in the controls; fatty acids 10.61 m eq., cholesterol 128 mg %, free cholesterol 33 mg %, lipid phosphorus 10.36 mg %.‖

Also the cocks which had been injected with the highest daily amounts of estrogen had light yellow or ochre-colored livers, presumably with a high fat content. Their kidneys were enlarged, pale and had an irregular surface, in some instances with cyst-like protrusions. In three of the treated cocks an aneurysm of the vena iliaca externa was observed.

The normal and Creeper cocks showed no difference in response. The treated capons showed the same blood and skeletal changes observed in the treated roosters.¶

§ Serum calcium methods used described in 1 above.

3 See also Lorenz, F. W., Chaikoff, I. L., and Entenman, C., *J. Biol. Chem.*, 1938, **126**, 763.

‖ Methods used for lipoid determinations are described in *J. Biol. Chem.*, 1932, **99**, 43; 1933, **101**, 695; 1937, **117**, 183; and 1937, **122**, 77; *Am. J. Physiol.*, 1938, **122**, 73.

¶ Since the conclusion of our experiments Zondek and Marx have reported calcemia and lipemia in young cockerels treated with estrogens. *Arch. Internat. Pharm. Dynam. and Therap.*, **61**, 11.

10579

Icteric Index in the Newborn.

Samuel B. Weiner and Miriam Reiner. (Introduced by Harry Sobotka.)

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The icterus index and the van den Bergh reactions of 49 specimens of cord blood and 50 sera of normal new-born infants were studied in an effort to determine the type of icterus in icterus neonatorum.

In unhemolysed sera Newburger[1] showed that the icteric index by the acetone method of Ernst and Förster[2] gave values approximately one-third those obtained by Meulengracht.[3] When hemolysis was present the icteric index values by acetone and water methods showed wide discrepancies. Every precaution was taken to prevent hemolysis but this was present in 30% of our specimens, probably due to the polycythemia and greater fragility of red cells in the new-born.

All of the infant sera were taken from normal healthy infants, 90% of whom were negroes and varied in weight from 5 to 9 pounds. The bloods were drawn from the deep jugular vein and were taken between the third and sixth day after birth when the icterus is at its highest level. The cord bloods were collected in wide mouth test tubes immediately after birth from the placental end of the severed cord.

In all of the non-hemolysed specimens, in cord blood, as well as in blood from the new-born, the icteric index values determined by water and acetone methods were equal. (Table I.) In the hemolysed specimens (Table II) slightly higher values were obtained by the water method, although the differences were much smaller than those obtained by Newburger in his series of cases.

The direct van den Bergh reaction was *negative* in all cases even when the amount of circulating bilirubin was very high. We obtained essentially the same ratio between milligrams of bilirubin determined by the indirect van den Bergh, and the icteric index by the acetone method, as Newburger found in his series. In all except 2 infants, hyperbilirubinemia was present (icteric index higher than 5 units by the acetone method). Among the cord bloods there was

1 Newburger, R. A., *J. Lab. Clin. Med.*, 1937, **22**, 1192.

2 Ernst, Z., and Förster, J., *Klin. Wchnschr.*, 1924, **3**, 2386.

3 Meulengracht, E., *Arch. f. Klin. Med.*, 1920, **132**, 285.

TABLE I.
Non-hemolysed Specimens.

Icteric Index Units Cord (29 cases)		Icteric Index Units Infant (35 cases)	
Water	Acetone	Water	Acetone
6	6	4	4
7.5	6	6	4
7.5	7.5	10	7.5
8	6	10	10
8	8	12	12 (2×)
10	10 (4×)	14	12
12	10 (4×)	15	12.5
12	12 (8×)	15	14
12.5	10	17.5	17.5
14	14	20	16
15	14	20	20
15	15 (3×)	24	22
16	14	27	25
18	18	27.5	27.5
		28	28
		30	25
		30	30 (2×)
		32	28
		36	36 (2×)
		39	36
		40	39
		45	45 (2×)
		45	40
		50	40
		52	45
		55	50
		75	70
		75	75
		96	90
		112	90
Avg Ratio: $\frac{\text{Water}}{\text{Acetone}} = 1.06 \pm 0.02$		Avg Ratio: $\frac{\text{Water}}{\text{Acetone}} = 1.09 \pm 0.02$	

only one specimen which showed a normal amount of bilirubin.

In the few cases of congenital hemolytic icterus in adults which we have studied to date we obtained similar results to those in the new-born; no direct van den Bergh reaction and identical icterus values by the 2 methods.

There was no direct or linear correlation between the icteric index of the cord blood and the birth weight or length of the infants; nor between the height of the infants' icteric index and the birth weight. In this relation it was interesting to note that in a set of identical twins with only a few ounces difference in weight, the icteric indices were 20 and 55 units respectively. However, in our series, 4 small infants weighing under 6 pounds had an average index of 46, whereas in 5 large infants over 8 pounds, the average was only 19.6 units. This is in agreement with the clinical observation that usually small

TABLE II.
Hemolysed Specimens.

Icteric Index Units Cord (20 cases)			Icteric Index Units Infant (15 cases)		
Water	Acetone		Water	Acetone	
10	5		8	5	
10	6		10	5	
12	6		14	10	
12	8		16	10	
14	8	(2×)	16	12	(2×)
15	7		17.5	10	
15	10	(2×)	20	10	
16	10	(2×)	24	18	
16	12	(4×)	24	20	
20	10		40	25	
20	12		40	35	
20	16		48	40	
20	15	(2×)	60	50	(2×)

Avg Ratio: $\frac{\text{Water}}{\text{Acetone}} = 1.6 \pm 0.06$ Avg Ratio: $\frac{\text{Water}}{\text{Acetone}} = 1.46 \pm 0.07$

infants become intensely jaundiced while few large infants show any clinical signs of icterus.

That bilirubinemia is present at birth[4, 5] has lately not been sufficiently stressed. Schick[6] pointed out that this was related to the destruction of maternal red cells into an iron-containing fraction and bilirubin. The iron is utilized by the foetus while the bilirubin is excreted via the placental circulation.

Summary. 1. The icteric index determined by the water and acetone methods gave equal values in a series of 49 normal cord bloods and 50 normal new-born infants. 2. Hyperbilirubinemia was present in all but one of the cord bloods and 2 of the new-born infants. 3. All specimens gave a negative direct van den Bergh reaction even when the sera showed marked hyperbilirubinemia. 4. In several cases of congenital hemolytic icterus the same findings were present: the direct van den Bergh was negative and the icteric index gave the same values by both the water and acetone methods. 5. These methods may be used to differentiate hemolytic icterus from other types of icterus. The results support the hemolytic theory of icterus neonatorum.

4 Hirsch, A., *Z. f. Kinderh.*, 1913, **9**, 196.

5 Yllpö, A., *Z. f. Kinderh.*, 1913, **9**, 208.

6 Schick, B., *Z. f. Kinderh.*, 1920, **27**, 231.

10580

Effect of Partial Clamping of Aorta in Dogs upon Diastolic Pressure in Carotid and Femoral Arteries.

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It is well known that in the human congenital anomaly, coarctation of the aorta, both *systolic* and *diastolic* levels of pressure are frequently elevated in the arteries proximal to the constriction.[1, 2] It has recently been suggested that interference with the blood supply to the kidneys is the mechanism underlying the development of the arterial hypertension found in this condition.

The evidence that this is so is that arterial pressure rises in the upper half of the body only when the clamp upon the aorta is placed above the site of origin of the renal arteries. Rise in mean pressure in the carotid artery was studied by direct measurements in dogs[3] and increase in pressure in rats[4, 5] was inferred from the weights of the hearts at death being distinctly greater when the aortas were clamped above the origin of the renal arteries than when they were clamped below or not clamped at all.

If the mechanism of the development of hypertension occasioned by clamping the aorta resembles that which follows clamping the renal arteries there should occur an increase in diastolic arterial pressure throughout the body in parts distal, as well as proximal to, the constriction of the aorta. So far, diastolic pressure has not been measured in experimental work nor were pressures in the hind extremities measured. Yet in human cases of coarctation of the aorta collected by King[2] the data suggest and, more recently direct measurement of pressure in the femoral artery in a case[6] shows, that elevation of diastolic pressure may be present in the lower extremities.

In the present study observations were made of the changes in diastolic pressure in the legs of three dogs following partial clamping of the aorta above the origin of the renal arteries. The experiments were carried out as follows: Van Leersum loops[7] were made of one

1 Lewis, T., *Heart*, 1933, **16**, 205.

2 King, J. T., *Ann. Int. Med.*, 1937, **10**, 1802.

3 Goldblatt, H., and Kahn, J. R., Proc. Cent. Soc. Clin. Res., *J. Am. Med. Assn.*, 1938, **110**, 686.

4 Rytand, D. A., Proc. Soc. Exp. Biol. and Med., 1936, **38**, 10.

5 Rytand, D. A., *J. Clin. Inv.*, 1938, **17**, 391.

6 Steele, J. M., and Cohn, A. E., *J. Clin. Inv.*, 1938, **17**, 514.

7 Van Leersum, E. C., *Arch. ges. Physiol.*, 1911, **142**, 377.

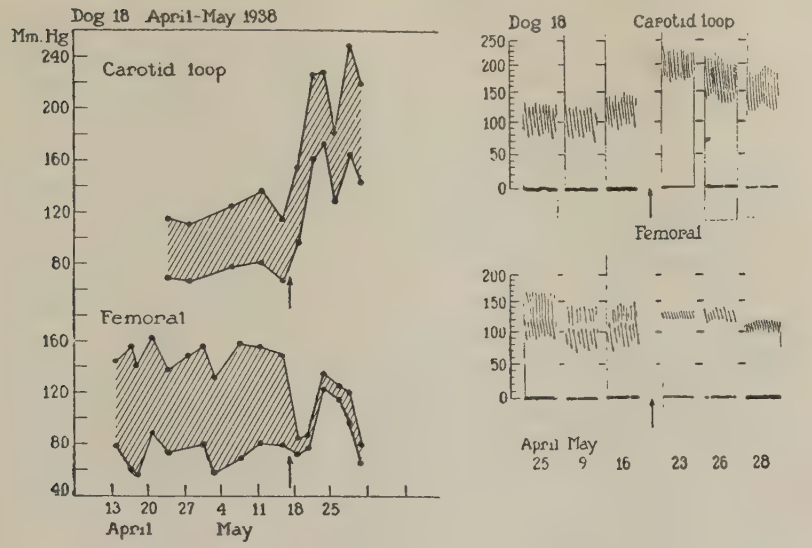

FIG. 1.

Chart of carotid and femoral arterial pressures from dog No. 18 read from records obtained by use of Hamilton's intra-arterial manometer. At the right (Fig. 1a) are reproduced a few of the original records. The black arrows indicate application of the aortic clamp on July 7.

carotid artery. Pressures were then recorded in both the carotid and femoral arteries by means of Hamilton's intraärterial manometer[8] about 4 times a week. The frequency and sensitivity of the manometer were, of course, such that both systolic and diastolic levels of pressure were accurately recorded. Relatively constant levels became established in 3 or 4 weeks. The animals were then anesthetized with pentobarbital and an adjustable metal clamp was placed upon the aorta above the renal arteries, but below the coeliac axis and adrenal arteries. Some interference with the adrenal blood supply occurred, in all probability, in most of the animals but it was obviously not sufficient to interfere perceptibly with the function of the glands. The aorta was gradually compressed by means of the clamp while pressure in the femoral artery was continuously recorded until pulsation had almost disappeared. The clamp was then fixed in this position. At this point little fall in diastolic level occurred, but if an attempt was made to obliterate the pulse altogether, the diastolic pressures fell abruptly. After securing the clamp the wound was closed. Records of pressure were obtained after operation almost daily for a period of about 2 weeks.

Within 24 hours both systolic and diastolic pressures in the caro-

8 Hamilton, W. F., Woodbury, R. A., and Harper, H. T., *Am. J. Physiol.*, 1934, **107**, 427.

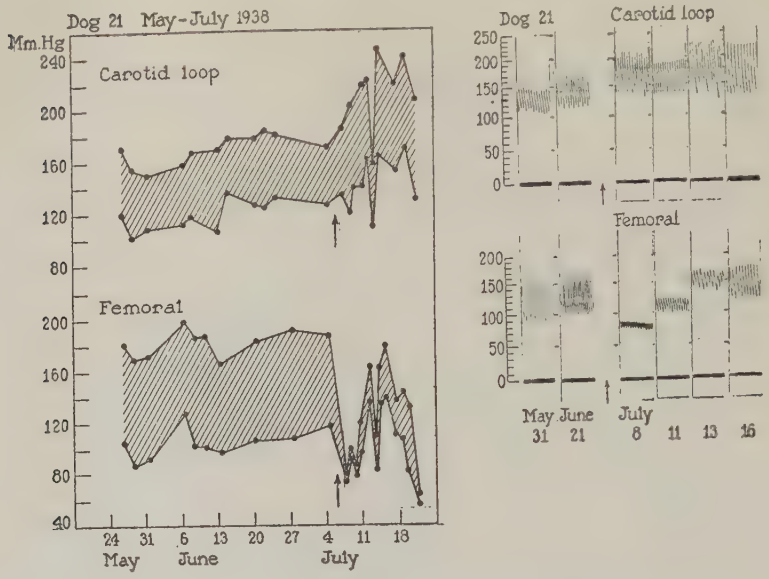

FIG. 2.
Same as Fig. 1, for dog No. 21. The clamp was applied on May 17.

tid artery rose (Figs. 1 and 2). During the next 72 hours both levels of pressure in the femoral artery began also to rise from the very low postoperative levels occasioned by constriction of the aorta, and the pulse pressure, though still small, increased. Within 5 days the *diastolic* pressure in the lower extremities rose to a level plainly higher than the preoperative one and remained elevated for about a week, or until slow hemorrhage into neighboring tissues took place through erosion of the aorta by the clamp.

The diastolic level in the hind legs does not, of course, always rise. Whether it does seems to depend upon the degree of occlusion of the aorta. If the aorta is completely occluded, and if sufficient collateral circulation fails to develop, as occurred in one instance, the pressure in the hind legs remains low though both pressures rise in the carotid artery; but if it is insufficiently clamped neither the pressures in the legs nor those in the carotid artery rise. The marked reduction in flow to the lower extremities seemed in the instance of complete occlusion responsible for the failure of the pressure to rise; the hind limbs were cold and failure of nutrition began before the animal was killed.

From these experiments the conclusion can be drawn that clamping the aorta in dogs above the orifices of the arteries to the kidneys may be followed by diastolic hypertension in the hind legs as well as in the neck. Increase in peripheral resistance is widespread. Elevation of

pressure does not depend therefore upon mechanical factors alone as in acute experiments when the aorta is occluded (Barcroft[9]). Nor can the hypertension in coarctation of the aorta be explained by local mechanical factors such as the increase in resistance offered by the narrowed aorta and the collateral paths around it.[10] It depends upon a reaction of the whole peripheral arteriolar system. Hypertension the result of clamping the aorta is in this respect similar to that following constriction of the renal vessels. In both cases the deciding factor is interference with the dynamics of the renal circulation.

One further remark seems pertinent. The observation of similar consequences to the arterial pressures of constriction of the aorta in man (coarctation of the aorta) and in dogs (clamping of the aorta) warrants the inference that interference with the hemodynamics of the renal blood supply in man may lead to arterial hypertension as Goldblatt has demonstrated that it does in dogs and monkeys.

Summary. Clamping the aorta above the orifices of the renal artery in dogs is followed by elevation of the diastolic level of arterial pressure in the hind legs as well as in the carotid arteries. Constriction of the peripheral arterioles is, therefore, a general phenomenon, just as when it follows partial clamping of the renal arteries. The hypertension which develops in coarctation of the aorta in man is on this evidence analogous to that which accompanies constriction of the renal arteries. The evidence suggests strongly that interference with the hemodynamics of the renal circulation leads to hypertension in man as well as in animals.

10581

Form of Ventricular Contraction in Cardiac Infarction; Fluoroscopic Studies.

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Infarction of the heart following coronary artery occlusion is attended by a profound disturbance in the circulation. While the circulatory dynamics and electrocardiographic changes have been

9 Barcroft, H., *J. Physiol.*, 1931, **71**, 281.

10 Blumgart, H. L., Lawrence, J. S., and Ernstene, A. C., *Arch. Int. Med.*, 1931, **47**, 806.

extensively investigated, very little attention has been paid to direct observation of alterations in cardiac contraction.

That experimental ligation of a coronary artery interferes with the orderly contraction of the ventricles has been known since the 17th century.[1, 2] A detailed description of the changes in ventricular contraction, however, has been lacking until the recent investigation of Tennant and Wiggers.[3] Employing the myocardiograph, these authors found that immediately after occluding branches of the coronary arteries in dogs the area of muscle rendered ischemic ceased to contract and paradoxical (reverse) movements occurred, *i. e.*, the ischemic area bulged passively while the remainder of the ventricle contracted normally.

There have been few recorded fluoroscopic observations in man of the effect of coronary artery occlusion on the contractile movements of the heart, even in cases where the infarct is so extensive as to result in the formation of a ventricular aneurysm.[4, 5, 6] Zadek,[7] and Levene[8] observed localized impairment of contraction of the left ventricle in coronary artery disease but did not describe systolic expansion (reversal of pulsation) which was a characteristic finding in the studies of Tennant and Wiggers on dogs.

The movements of the left ventricle in 64 cases of myocardial infarction due to coronary artery occlusion have been studied by

TABLE I.
Types of Ventricular Contraction in Myocardial Infarction.

	Fluoroscopy	Roentgenkymography
1. Normal pulsation	17	13
2. Reversal of pulsation		
a. complete reversal	26	24
b. partial reversal		
lag of systolic contraction	4	4
doubling of systolic contraction	2	5
3. Impairment of contraction		
a. absence of pulsation	2	5
b. diminution of pulsation	13	13
Total	64	64

1 Chirac, P., *De Motu Cordis, Adversaria Analytica*, 1698, p. 121, cited by Sée, Bochefontaine and Roussy, *Compt. Rend. Acad. d. Sc.*, 1881, **92**, 86.

2 Samuelson, B., *Z. f. Klin. Med.*, 1881, **2**, 12.

3 Tennant, R., and Wiggers, C. J., *Am. J. Physiol.*, 1935, **112**, 351.

4 Sezary, A., and Alibert, J., *Bull. et mem. Soc. med. d. hop. de Paris*, 1922, **46**, 172.

5 Lenk, R., *Fortschr. a. d. Geb. d. Röntgenstrahlen*, 1926, **35**, 1265.

6 Kalisch, Z., *Wien. Klin. Wchnschr.*, 1927, **40**, 1078.

7 Zadek, E., *Klin. Wchnschr.*, 1932, **11**, 1255.

8 Levene, G., Lowman, R. M., and Wissing, E. G., *Am. Heart J.*, 1938, **16**, 133.

TABLE II.
Regions of Left Ventricle Exhibiting Abnormal Contraction.

	Fluoroscopy	Roentgenkymography
Normal	17	13
Upper section left ventricular contour	3	2
Middle " " " "	6	3
Supraäpical " " " "	8	13
Apical " " " "	11	12
Lower half " " "	16	18
Entire " " "	3	3
Total	64	64

fluoroscopy at intervals varying from one month to 4 years after the acute attack. The findings were compared with roentgenkymograms which were taken immediately after fluoroscopy. In addition cine-roentgenographic studies were carried out in a group of 24 subjects with normal hearts and with cardiac infarcts.

The fluoroscopic observation was performed with the patient in the postero-anterior position, visualizing the anterior wall and apex

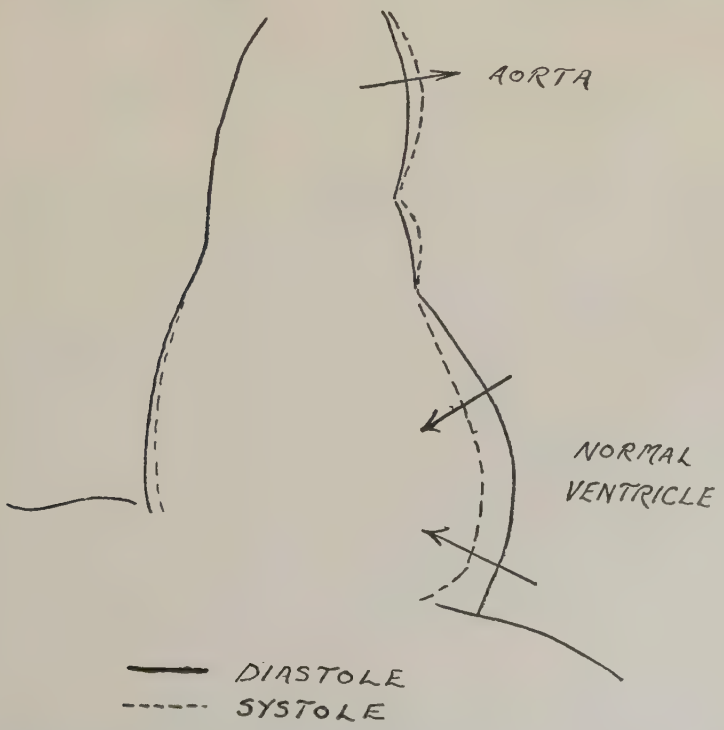

FIG. 1A.

Normal Contraction. Entire left ventricle contracts during systole. There is an inthrust of the ventricle synchronous with expansion of the aorta.

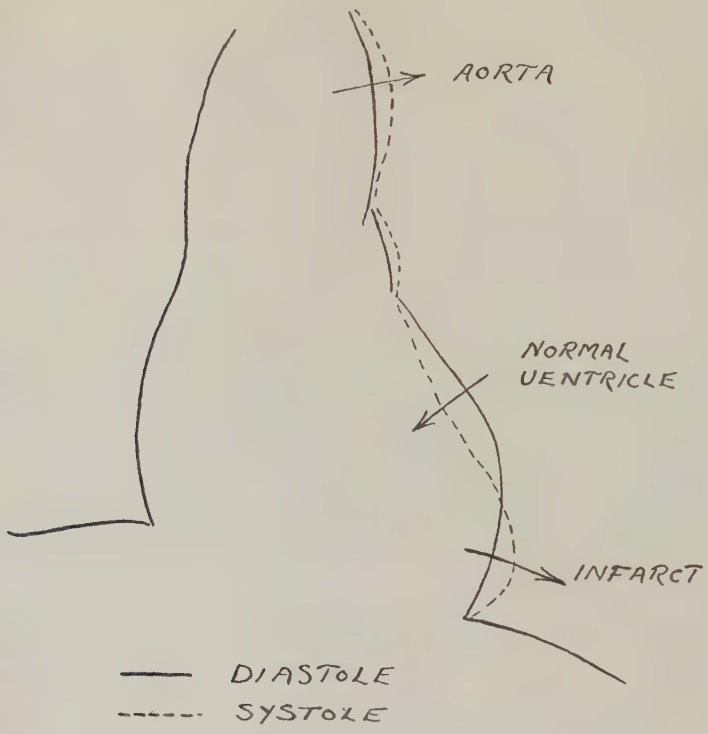

FIG. 1B.

Reversal of Contraction. While the upper normal portion of the left ventricle contracts (inthrust), the diseased lower apical muscle is passively expanded (outthrust).

of the left ventricle. Time relationships of the pulsations were established by comparing the movements of the left ventricle with those of the aorta and pulmonary artery. The arterial pulsations are normally in opposite phase to those of the ventricle, the aorta expanding in systole while the ventricle contracts (Fig. 1A). Another method for establishing time relationships was auscultation of the heart by means of a Bowles diaphragm held to the patient's chest at the left costal margin in the fourth interspace. Normally there is observed an inthrust of the entire left ventricular contour and elevation of the apex and diaphragmatic surface of the heart synchronous with the expansion of the aorta and the first heart sound (Fig. 1A).

The contractile movements of the heart were studied at the end of a deep inspiration. In addition to immobilizing the diaphragm this procedure also slows the heart rate. This is of advantage since rapid heart action makes visualization of the details of ventricular move-

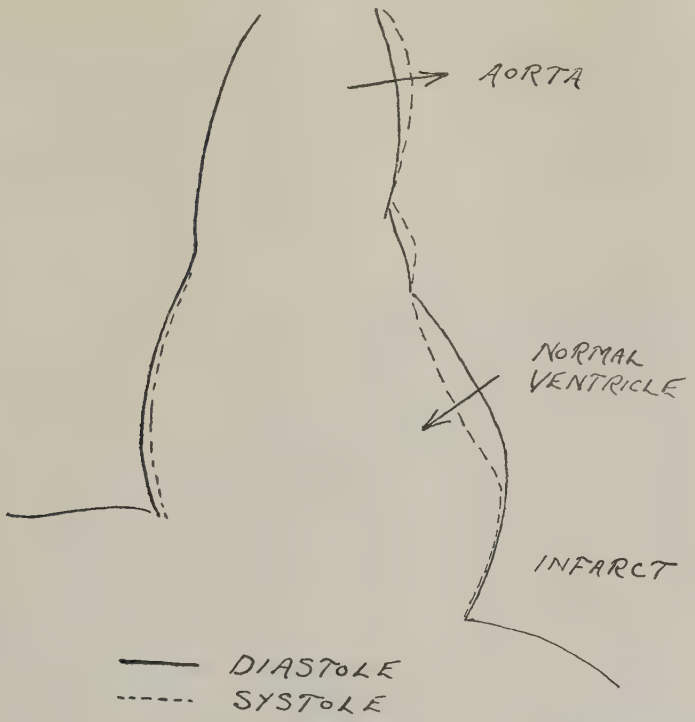

FIG. 1C.

Absence of Pulsation. While the normal upper portion contracts (inthrust), no movement is seen in the affected lower half.

ment difficult. It was also found helpful to magnify the movements by drawing the screen away from the patient, particularly when they were of small amplitude. The presence of apical pericardial fat may obscure muscle movement and give the impression of diminution or absence of pulsation. One must therefore look within the fat pad to observe muscle contraction.

Abnormalities in contraction were observed on fluoroscopy in 47 of the 64 cases studied (73%). The types of movement observed are classified in Table I. The fluoroscopic observations corresponded closely with roentgenkymographic findings both in regard to the type of movement (Table I) and the region of the left ventricle involved (Table II). The lower half of the left ventricle, particularly the apical and supraäpical segments, most frequently exhibited these abnormalities in contraction.

Reversal of pulsation was the most common and definite abnormality of ventricular contraction associated with myocardial infarction (50%). It frequently appeared as a wavelike movement

along the border of the left ventricle. As the intraventricular tension abruptly rose in the isometric phase of systole the weakened myocardium at the site of the infarct passively expanded (outthrust), while the normal region of the ventricle contracted (inthrust) (Fig. 1B). This phenomenon was also observed in 3 cases of ventricular aneurysm. Occasionally the reversal of pulsation was not complete but appeared as a definite lag of systolic inthrust or as a double systolic pulsation.

Localized impairment of contraction was observed in 23% of the cases. This appeared as a marked diminution or absence of pulsation (Fig. 1C). These changes may occur in a region where the infarcted myocardium is not sufficiently powerful to contract vigorously but is able to withstand the intraventricular pressure.

These localized abnormalities in pulsation have not been observed by us in normal individuals or in those with other types of heart disease than coronary artery disease. The pulsations are occasionally much diminished, or even absent, in the apical region when the heart is markedly enlarged, but such cases were not included in the present series.

In this report we have presented the abnormalities in contraction of the left ventricle as observed in the postero-anterior view alone. In addition, the movements of the postero-lateral wall of the left ventricle in myocardial infarction are being studied in the left oblique and left lateral positions.

10582 P

Basal Heat Loss and Production in Women at Temperatures From 23°C to 36°C.

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Recent work[1, 2, 3] on the effect of environmental temperature on heat loss in humans has been restricted to male subjects, most of them

1 Hardy, J. D., and Du Bois, E. F., *J. Nutrition*, 1938, **15**, 461.

2 Winslow, C.-E. A., Herrington, L. P., and Gagge, A. P., *Am. J. Physiol.*, 1937, **120**, 1.

3 Hardy, J. D., and Soderstrom, G. F., *J. Nutrition*, 1938, **16**, 5, 493.

TABLE I.

Subject Symbol	Age	Height, cm	Weight, kg	Surface Area, m^2
○	35	175	64.0	1.77
△	24	162	59.5	1.62
□	26	165	54.5	1.60

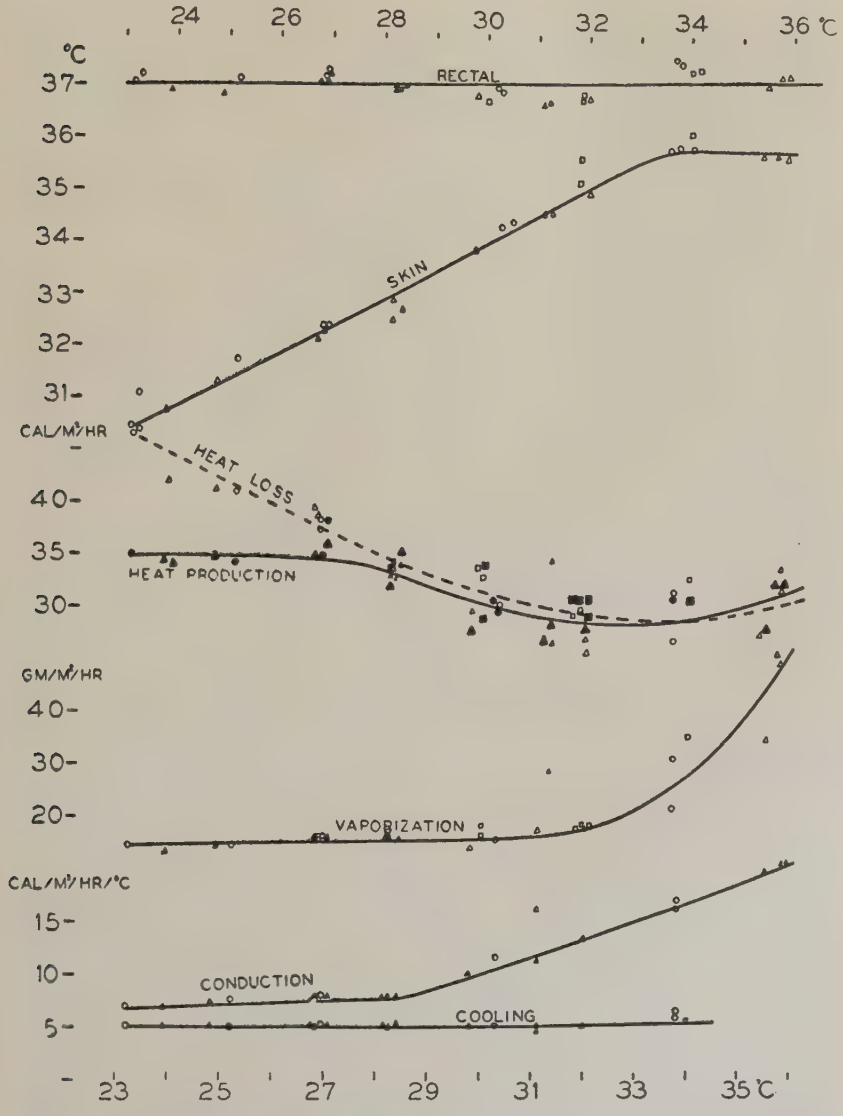

FIG. 1.

Experimental data for three subjects plotted against calorimeter temperature. (Solid symbols: Heat production.)

nude. The present report deals with similar studies on 3 normal women (nude). The methods were similar to those previously described.[1]

The data are presented in Fig. 1, in which all variables are plotted as functions of the calorimeter temperature.

1. *Rectal Temperature* was about 0.15°C lower at 24°C than at 36°C.

2. *Average Skin Temperature* increased linearly with calorimeter temperature between 23°C and 33°C. These changes in skin temperature were due entirely to changes in the calorimeter temperature. At 33°C the skin temperature reached a high level and did not change with further increase in calorimeter temperature.

3. *Heat loss* decreased linearly with increasing calorimeter temperatures up to 31°C, and after reaching a low point of 29 cals/m²/hr at 32°C rose slowly with higher temperatures.

4. *Heat production* was constant between 23°C and 27°C at 35 cals/m²/hr. At 28°C, the heat production began to decrease slowly, closely following the heat loss. At 32°-33°C a low point in heat production, 29 cals/m²/hr was reached after which the heat production again increased following closely the heat loss. This change in heat production was observed in all 3 subjects and amounted to over 17% of the 35 calorie level.

5. *Vaporization* was practically constant between 23°C and 32°C. Sweating began between 32°C and 33°C. In this zone evaporation was that necessary to balance heat loss and heat production.

6. *The conduction* of the peripheral tissues was almost constant between 23°C and 28.5°C, and had a value corresponding to a thickness of tissue of 22 mm. The conduction increased linearly with temperature from 28.5°C to 36°C. Thus blood flow in the superficial tissues increased in proportion to the rise in environmental temperature.

7. *The cooling constant* of Newton's law was 5.3 cals/m²/hr/°C and did not change throughout the experimental range. The constancy of this figure is a check on the heat loss, calorimeter temperature, skin temperature, and amount of activity of the subject.

Fig. 2 shows a comparison between the present data and those previously reported on male subjects. While the rectal temperatures and the Newton's law cooling constants were identical, there were significant differences in the responses of the men and women to temperature changes.

The skin temperature of the women was higher in the warm atmosphere and lower in the cold than that of the men. The dif-

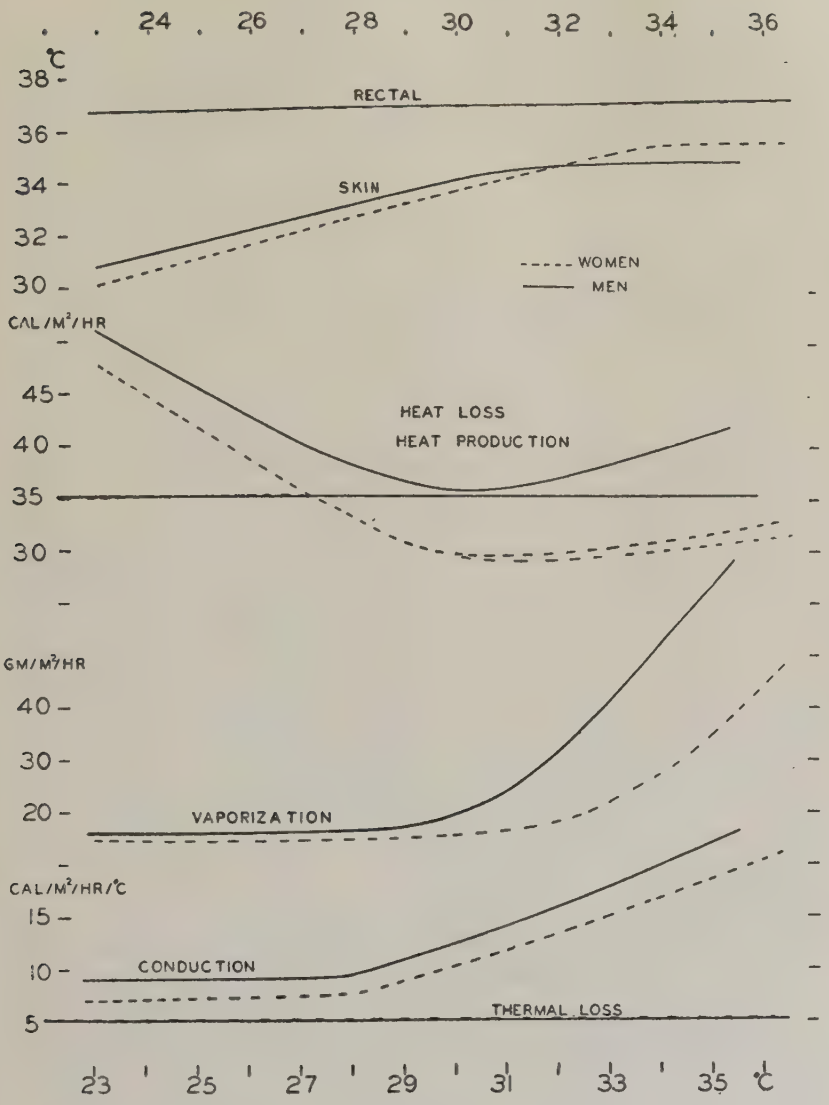

FIG. 2.
Comparison of responses of men and women to environmental temperature.

ference at the higher temperature is accounted for both by the level of skin temperature required to induce sweating and by the amount of sweat. The women did not begin to sweat until the calorimeter temperature was 2 degrees above the threshold for sweating in the men, and the amount of sweating was less. The lower skin temperature of the women in the cold is apparently due to a thicker insulating layer of superficial tissue. This view is verified by the

differences in the heat loss per unit area and in the tissue conduction. The heat production for the men and women was the same up to 27.5°C, but in contrast to the men, the women showed a significant decrease in heat production at temperatures above this level.

10583

Quantitative Study of Effect of Transfusion of Blood on Plasma Prothrombin.*

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It is established that the bleeding tendency in obstructive jaundice and biliary fistula is due to a lowering of the plasma prothrombin. Both on clinical[1] and experimental[2] grounds, blood transfusion is known to effect temporary improvement in the hemorrhagic state. Warner, Brinkhous and Smith[2] have shown in dogs with a biliary fistula of long duration that after a blood transfusion there is a temporary rise in plasma prothrombin with a cessation of bleeding.

The purpose of this report is to demonstrate in dogs that it is possible to determine quantitatively the prothrombin change which occurs following transfusion and that the change is purely one of summation.

The animals used as recipients were of 3 types: 2 animals were normal dogs, 2 were dogs with obstructive jaundice, and one was a dog with a cholecyst-nephrostomy.[3] Normal dogs were used as donor animals. Plasma volumes before transfusion were determined by the vital red method.[4] The plasma volume after transfusion was calculated by addition of the volume of transfused plasma to the pre-

* This study was carried out under a grant from the John and Mary R. Markle Foundation.

1 Judd, E. S., Snell, A. M., and Hoerner, M. T., *J. Am. Med. Assn.*, 1935, **105**, 1653.

2 Smith, H. P., Warner, E. D., Brinkhous, K. M., and Seegers, W. H., *J. Exp. Med.*, 1938, **67**, 911.

3 Kapsinow, R., Engle, L. P., and Harvey, S. C., *S. G. and O.*, 1924, **39**, 62.

4 Rowntree, L. G., and Brown, G. E., *The Volume of the Blood and Plasma*, W. B. Saunders Company, 1929.

TABLE I.

	Recipient			Donor					
Dog No.	Plasma vol., cc	Prothromb. units, per cc	Total units	Vol. transfused plasma, cc	Prothromb. units, per cc	Total units transfused plasma	Expected Total prothromb. units	Actual Total prothromb. units	% difference
1. P-1	386	30	11580	69	74	5106	16686	17745	+6.3
2. P-2	300	65	19500	69	89	6141	25641	24354	—5.0
3. P-1	430	75	32250	75	89	6675	38925	38885	—0.1
4. P-15	715	106	75790	78	108	8424	84214	81679	—3.0
5. P-10	860	39	33540	95	100	9500	43040	44862	+4.2
6. P-15	850	72	61200	94	116	10940	72104	66080	—8.4
7. P-17	925	64	59200	115	107	12305	71505	69160	—3.3

transfusion value. Prothrombin units before and 3 hours after the transfusion were determined by the method of Warner, Brinkhous and Smith.[5] All transfusions were performed by the indirect method, using sodium citrate as the anticoagulant.

The experiments in Table I show the effect of transfusions of citrated blood. Within the limits of experimental error, the actual total prothrombin content of the plasma of the recipient determined three hours after transfusion corresponds closely with the expected total. The error varies from plus 6.3% to minus 8.4% with an average of minus 1.3%. This indicates that the prothrombin in the plasma of the donor is distributed throughout the plasma of the recipient and can be detected quantitatively in the latter 3 hours after the transfusion. It further indicates that the change following transfusion is simply one of summation.

In vitro tests were also carried out. Blood was drawn from a normal dog and from a deficient dog. The plasma prothrombin content was determined in the 2 samples of blood and in differing mixtures of the two. For example, .8 cc of plasma having a prothrombin content of 107 units per cc was mixed with .2 cc of plasma having a prothrombin content of 25 units per cc. The expected prothrombin units of the 1 cc of mixed plasma would therefore total 91 units. Actually, the mixture when tested showed 99 units, a percentage difference of 9%. Various mixtures of the above plasma and of other plasmas treated in the same way showed percentage differences which ranged from minus 29% to plus 15%.

Summary. The changes in the total content of prothrombin in the plasma of a recipient after a transfusion are dependent on the prothrombin content of the plasma of the donor, and may be calculated on the basis of addition.

[5] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

10584

Failure of Gonadotropic Function of the Rat Hypophysis During Chronic Inanition.*

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The disease picture of anorexia nervosa with marked weight loss has many features similar to that of true hypophyseal destruction in Simmond's disease. The similarity of the syndromes raises the possibility that the reduced food intake in anorexia nervosa may cause hypophyseal failure. This is further suggested by the amenorrhea that occurs with starvation and chronic wasting disease.

To test this hypothesis, the effect of inanition on hypophyseal function has been studied in the rat during the past 2 years. Marrian and Parkes,[1, 2] in investigating the anestrus of vitamin B deficiency in the rat, found that the ovaries of such rats were still responsive to hypophyseal implants, as were also the ovaries of rats on a severely diminished food intake. This latter has been recently confirmed by Mulinos, *et al.*[3] The presence of gonadotropic principle in the hypophysis of the vitamin B deficient rats, however, led Marrian and Parkes[1] to believe that the rôle of the hypophysis in the anestrus in this condition was not clear. The experiments described below indicate plainly that during chronic inanition, the origin of the anestrus is in the anterior hypophysis.

Adult female rats, 180-260 g in weight, with regular estrous cycles, and immature 22-day-old female rats of the Long-Evans strain, were used throughout. The rats were kept in individual cages, given water *ad lib.*, and were fed a standard rat diet, McCollum mixture No. 1, limited only in amount. Cod liver oil and yeast supplements were given twice a week. The rats were used after 1-4 months on this regime, having lost 30%-50% of their original body weight. Swiss mice, 22 days old at weaning, were used as recipients for the hypophyseal implants, with autopsy 96 hours after a single

* Aided by a grant administered by P. E. Smith, from the Rockefeller Foundation, New York City.

† Assigned to Department of Neurology.

1 Marrian, G. F., and Parkes, A. S., *Proc. Roy. Soc. B*, 1929, **105**, 248.

2 Parkes, A. S., *Quart. J. Exp. Physiol.*, 1928, **18**, 397.

3 Mulinos, M. G., Pomerantz, L., Smelser, J., and Kurzrok, R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 79.

implant, using the technic of Smith. Tissues were fixed and stained by the usual technics.

I. The effect of inanition on the structure and function of the ovary has been previously reported[4, 5, 6] and has been confirmed here in a small series. However, the microscopic appearance of the ovary can now be identified as that occurring after partial hypophysectomy. In the present series, the estrous cycle, ovarian and uterine weights were observed. Sixty-one of 75 adult rats showed a diestrous smear after 2 weeks, and 71 of the 75 after 3 weeks of inanition with no reappearance of estrus except in 4 rats, in 1 of which cycles never ceased despite pronounced weight loss. Four adult rats were autopsied after 1 month of inanition. Ovarian weights ranged from 26.5-53.0 mg, averaging 29 mg. Five hypophysectomized rats of about the same weight, 1 month after the operation had ovarian weights of 14.2-25.4 mg, averaging 18.2 mg. The ovarian weights of both of these groups are well below those of normal controls. This is also true of the uterine weights, which ranged from 122-162 mg (av. 141) in the starved rats compared to 76-201 mg (av. 113) in 7 castrate starved rats and 76-111 mg (av. 88) in 5 hypophysectomized rats. The above figures in animals suffering from severe inanition approach those seen after almost complete ablation of the anterior hypophysis. The gross and microscopic appearance of the ovaries, and their response to gonadotropic principles are also like those of the partially hypophysectomized rat.

II. The responsiveness of the ovary during inanition was tested with the gonadotropic principles of pregnancy urine, anterior hypophysis, and pregnant mare's serum.‡

a. Eight starved rats, 3 adult and 5 immature, were injected with pregnancy urine principle (1.5-3 r.u. total). There was some growth of follicles, thus differing from the hypophysectomized rat. There was also an occasional formation of corpora lutea and thecal luteinization. The adults remained in estrus only 48 hours.

b. The hypophyseal principle was injected into 34 adult starved rats, a total dosage of 2-20 mg being administered in 2 equal daily injections. Estrous response appeared with 10 mg dosage and

[4] Jackson, C. M., *The Effects of Inanition and Malnutrition upon Growth and Structure*, P. Blakiston, Philadelphia, 1925, 616 pp.

[5] Papanicolaou, G. N., and Stockard, C. R., PROC. SOC. EXP. BIOL. AND MED., 1920, **17**, 143.

[6] Evans, H. M., and Bishop, K. S., *J. Met. Res.*, 1923, **3**, 233.

‡ The pregnancy urine extract, Follutein, was given by E. R. Squibb and Sons. The anterior hypophyseal extract was primarily thyrotropic and was provided by the Schering Corporation. The pregnant mare serum extract was Antex Leo.

lasted 1-2 days. Nineteen 22-day-old normal rats were given 2-40 mg in 5 doses over 36 hours and autopsied 96 hours after the first injection. Minimal ovarian and uterine weight response occurred with the 10 mg total dose. Ovaries averaged 18.2 mg, uteri 49.2 mg, compared with untreated control figures of 14.7 and 33.8 mg respectively.

c. Pregnant mare's serum gonadotropic extract (Antex) was injected into 47 adult starved rats using total doses of 0.05-0.8 mg (0.25-4.0 m.u.), and into 31 normal unstarved immature rats, 0.05-0.6 mg (0.25-3.0 m.u.) being used, and the duration of estrus observed. The material was given to the adults in 2 injections and to the immatures in 5 injections over a 2-day period. In the 11 normal immature rats receiving 0.6 mg (3 m.u.) total of extract, estrus appeared on day 4 and lasted 1 day, whereas in the adult starved rats given this dosage, estrus lasted 3-13 days, and in 10 trials on 8 hypophysectomized rats with the same dosage, it lasted for 6 days. The cessation of ovarian function during starvation therefore has been shown to occur in the face of normal or slightly increased reactivity of the ovaries to gonadotropic principles. Thus, inability of the ovary to react to gonadotropic principle from the hypophysis cannot be the cause of the failure of ovarian function during inanition. As regards duration of estrus induced by pregnant mare serum, the starved rats behaved like the hypophysectomized ones though we feel uncertain why estrus should be prolonged in either type.

III. The fact that the ovary is sensitive to the hypophyseal principle suggests that interference with hypophyseal function might be the cause of the ovarian inactivity. The hypophyses from 6 adult starved rats were examined. The histological appearance, with evidence of atrophy, described by Jackson,[4] was found. No castration changes were seen as would be expected were the ovarian failure primary and the hypophysis responding normally. Eleven rats were starved and castrated. One to 2 months after castration the hypophyses were examined. No castration changes were found in the glands of 8 of the 11. The glands were identical in appearance with those of the intact starved animals. The castration change was prevented from developing whether the operation was performed before or during the inanition. In the other 3 there were definite castration changes. The degree of change was somewhat less than in the normal fed castrate.

Following castration, there is normally a marked rise in the gonadotropic potency of the castration hypophysis.[7] Tests were

[7] Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101.

TABLE I.
Assay by Mouse and Uterus Weight of Gonadotropic Potency of Starved Rat Hypophyses.

Mouse Organ	Total Number of Donors Tested						
	Adult starved castrate	Adult normal castrate	Adult starved	Adult normal	Immature starved	Immature normal	Normal mice controls
Ovaries (wt mg)							
0.0-3.3	6	0	6	3	0	1	10
3.4-5.0	2	0	0	4	4	1	2
over 5.0	4	5	0	3	0	2	0
Range in wt	2.1-7.7 mg	5.5-11.7 mg	1.9-3.3 mg	1.4-6.9 mg	3.3-4.8 mg	2.4-5.6 mg	1.6-4.5 mg
Avg wt	3.9 mg	8.3 mg	2.6 mg	4.2 mg	3.7 mg	3.5 mg	3.0 mg
Uterus (wt mg)							
0.0-12.0	8	0	5	2	0	1	12
over 13.0	4	5	1	8	4	3	0
Range in wt	8.2-44.0 mg	23.4-50.2 mg	7.2-14.7 mg	9.1-51.6 mg	17.7-27.9 mg	7.6-46.6 mg	3.9-11.6 mg
Avg wt	18.4 mg	34.4 mg	9.8 mg	29.5 mg	21.7 mg	23.0 mg	6.8 mg
Vagina							
Closed	4	0	5	0			12
Partly open	2	0	0	0			
Open	6	5	1	6			

carried out to determine whether or not an increase in potency after castration occurred in the starved animals. The hypophyses of 6 intact starved and 12 starved rats, castrated for at least 1 month, were implanted into the thigh muscles of 22-day-old white mice, 1 hypophysis per mouse. Ten normal rats and 5 normally fed rats, castrated for 2 months, served as controls. The hypophyses of 4 immature starved, and 4 immature normally fed rats were also assayed (Table I). The mice were killed 96 hours after implantation. There was a marked decrease in the potency of the hypophyses of the intact starved rats compared with the normal controls. Eight of the 12 castrate, starved rats showed not only no increase in potency of the hypophysis but the same low content as the intact starved rats. The glands of the other 4 contained gonadotropic potency of the order of normal castrates. These results indicate a failure of the hypophysis during inanition to respond to castration. The findings of a rise in potency with castration in 4 of the 12 castrate starved rats agrees with the percentage of starved rats which showed castration changes structurally.

The evidence, then, reveals that the gonadotropic function of the hypophysis fails during chronic inanition in the rat. The ovaries shrink to a partially hypophysectomized level. They are still normally responsive to gonadotropic substances. The atrophic changes in the hypophysis described after starvation have been confirmed and associated with this is a failure of response to castration either histologically or by rise in gonadotropic titer. It seems not improbable that the same mechanism operates to produce amenorrhea in human inanition and in anorexia nervosa with marked weight loss.

The other functions of the hypophysis during chronic inanition are being investigated.

10585 P

Rôle of Pituitary Stalk in Regulation of Thyrotropic and Thyroid Activity.

Unto U. Uotila.* (Introduced by Joseph C. Aub.)

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Several features of the etiology and symptomatology of thyrotoxicosis have given rise to the assumption that the central nervous system somehow controls thyroid function, possibly through the pituitary stalk which transmits the diencephalo-hypophyseal system. Experimentally, after pituitary stalk section, irregular histological depression of thyroid function was found in rats by Westman and Jacobsohn,[1] whereas Brooks[2] failed to find any histological abnormalities in rabbits. In diabetes insipidus of rats the basal metabolism is normal accompanied by some enlargement of the thyroid (Swann and Johnson[3]).

In a previous study it was observed (Uotila[4]) that the thyroid stimulation caused by cold depends on an intact anterior pituitary. In a search for a possible nervous mediation of the cold stimulation of thyrotropic function, the rôle of the cervical sympathetics was studied. It was found that bilateral cervical sympathectomy causes a mild and temporary hypofunction of the thyroid. Sympathectomy also modifies the cold reaction of the thyroid, but does not prevent it. Since unilateral sympathectomy has no direct effect on the ipsilateral thyroid lobe at room temperature or after cold stimulation, it was concluded that the effect of bilateral sympathectomy is apparently mediated through the anterior pituitary. These experiments indicated that the sympathetics are not necessary for the continued maintenance of thyroid function and that the temporary changes following sympathectomy are promptly compensated for through some other pathway.

In a further search for the pathway mediating the cold reaction of the anterior pituitary and thyroid in rats the following studies were made: (1) the effect of pituitary stalk section on the thyroid

* Holder of a Finnish Government Fellowship (1938); Fellow of the Rockefeller Foundation (1939). This study was partially aided by a grant from the Josiah Macy, Jr., Foundation.

1 Westman, A., and Jacobsohn, D., *Acta pathol. et microbiol. scand.*, 1938, **15**, 435.

2 Brooks, C. M., *Am. J. Physiol.*, 1937, **119**, 280.

3 Swann, H. G., and Johnson, P. E., *Endocrin.*, 1939, **24**, 397.

4 Uotila, U. U., *Endocrin.*, 1939, in press.

at room temperature; and (2) the effect of pituitary stalk section on the cold reaction of the thyroid. The Cell Height Index (CHI) was used as an indicator of thyroid activity. CHI equals the average height of cells in approximately one hundred thyroid follicles. Similar methods have been approved as sensitive indicators of thyrotropic hormone (Uhlenhuth,[5] Rawson and Starr[6]). The pituitary stalk was cut under ether anesthesia by the parapharyngeal approach of Smith-Selye. The completeness of the stalk section was checked by serial sections.

In the first series (A) the rats were placed in the cold (5-6°C) for 7 days, beginning on the 46th day after operation. On the 53rd postoperative day all the rats were killed. Table I shows the results. In animals kept at room temperature after stalk section, the CHI was normal (+12.5%). Upon stimulation by cold, after complete stalk section, no appreciable increase in CHI was observed (+5.5%). However, in blank operation controls and in one animal where the pituitary stalk was incompletely cut, cold stimulation did cause an average of 60% increase in CHI.

In a second series of experiments (B), the rats were subjected to cold on the 14th postoperative day for 4 days. The results were

TABLE I.

Treatment	Series	No. of rats	CHI, avg and variations	Avg changes of CHI in %
Operative controls, room temp.	A	15	4.0 (3.1-7.3)	—
	B	7	3.0 (2.1-4.1)	—
Pituitary stalk section room temp.	A	5	4.5 (3.6-5.5)	+12.5
	B	5	3.1 (2.3-3.9)	+ 3.3
Blank control operation; A, 7 days; B, 4 days in cold	A	7	6.2 (4.9-7.6)	+56.0
	B	5	5.1 (3.0-6.3)	+88.0
Complete pituitary stalk section; A, 7 days; B, 4 days in cold	A	6	4.2 (3.7-4.9)	+ 5.5
	B	4	2.5 (2.1-2.8)	—17.0
Incomplete pituitary stalk section; A, 7 days; B, 4 days in cold	A	1	6.2	+55.0
	B	2	7.0 (6.4-7.6)	+133.0

5 Uhlenhuth, E., *Transact. Am. Assn. Goiter,* 1936.

6 Rawson, R. W., and Starr, P., *Arch. Int. Med.,* 1938, **61**, 726.

similar to those obtained in the first series. Five blank operation controls gave a CHI of +88% after cold; 4 complete stalk section animals gave a CHI of −17%, whereas 2 rats with incomplete stalk section, subjected to the same period of cold, exhibited a CHI of +133%.

The weights of adrenals and testicles were about normal, showing that the lack of thyrotropic and thyroid response is not due to a general pituitary insufficiency.

Summary. In rats with intact pituitary stalks, exposure to cold stimulates the thyrotropic function of the anterior pituitary and the thyroid gland. After pituitary stalk section, rats at room temperature produce enough thyrotropic hormone to keep the thyroid histologically normal. In rats similarly operated on, but exposed to cold, the thyroid reaction is lacking. It is concluded that the pathways in the pituitary stalk transmit impulses regulating the secretion of thyrotropic hormone in the emergency state of exposure to cold.

10586 P

Effect of Testosterone Propionate on Ovulation and Luteinization in the Rabbit.

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A number of observers have reported a luteinizing action of testosterone propionate on the ovaries of rats and mice. A recent report by Freed, Greenhill and Soskin[1] suggests that small doses cause suppression of follicle formation in mice and rats while large doses cause stimulation. Mazer and Mazer[2] consider duration of treatment the determining factor, short treatment producing stimulation and prolonged treatment depression. In monkeys and in women this hormone seems to have a depressing effect on the ovaries. It seemed of interest to determine therefore whether ovulation in the rabbit in response to pregnancy urine would be influenced by testosterone propionate. The controls have indicated something of the effect of this hormone alone on the rabbit's ovary.

1 Freed, S. G., Greenhill, J. P., and Soskin, S., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 440.

2 Mazer, M., and Mazer, C., *Endocrinol.*, 1939, **24**, 175.

Twelve adult female virgin rabbits were placed in isolated cages and fed well for 6 weeks. Nine were then given testosterone propionate in oil* intramuscularly in 10 mg doses daily. After 22 days urine from a patient with a normal 5 months' pregnancy was given intravenously. Three days later laparotomy was performed in all 12 rabbits with excision of one ovary and cornu, and the treatment with testosterone continued. Autopsies were performed 15 days after the injection of pregnancy urine. The significant procedures and results are summarized in Table I.

TABLE I.

No. of rabbits	Testosterone Propionate mg daily	Pregnancy Urine (22d day) cc	Laparotomy (25th day)	Autopsy (37th day)
6	10	10	Multiple ovulations	Minute, pale corpora lutea
3	10	0	Small, pale ovaries	Small, pale ovaries
3	0	10	Multiple ovulations	Large yellow corpora lutea

In response to pregnancy urine multiple ovulations occurred in every case, both in the testosterone-treated animals and in the controls (2 to 9 blood points in each case). The size and bluish appearance of the uteri showed such variation as is common in the Friedman test. Microscopic examination of ovaries and uteri also failed to show a significant difference in the treated and untreated groups. The control animals which received testosterone propionate alone showed uniformly small, pale ovaries with small, pale uteri.

At autopsy 15 days after the injection of pregnancy urine the testosterone-treated animals showed uniformly very small, pale corpora lutea in contrast to the untreated animals which showed quite large, yellow corpora lutea. Histologically the corpora of the treated animals appeared to be considerably more degenerated than those of the untreated group, with small, atropic, partially vacuolated cells. In this short series no marked effect was seen on the ovaries of the animals receiving testosterone alone.

These data show that testosterone propionate in daily doses of 10 mg for a period of 22 to 37 days is without marked stimulating action on the rabbit's ovary; it does not prevent ovulation from the pregnancy urine anterior pituitary-like hormone or the formation of young corpora lutea. The data suggest, however, that in these dosages testosterone may accelerate degeneration of the corpora.

* For a generous supply of testosterone propionate we are indebted to Dr. R. MacBrayer of the Ciba Pharmaceutical Company.

Makepiece, Weinstein and Friedman[3] report that progestin inhibits ovulation in rabbits after mating, but does not prevent ovulation from the anterior pituitary-like factors of pregnancy-urine. In view of the similarity of action of progesterone and testosterone on the uterus it would be of interest to know if testosterone prevents post-coitus ovulation in the rabbit.

Our data on degeneration of the corpora lutea under testosterone confirm the findings of Courrier and Gros[5] who found that if the rabbit is injected with 10 mg of the propionate for the first 8 days of gestation, nidation does not occur and the corpora lutea rapidly degenerate, becoming small, pale and deep in the ovary. This action may explain the abortifacient effect of the male hormone in rabbits reported by Skowron.[4]

These findings on degeneration of the corpus luteum, if corroborated in the human, might have some clinical application. Many cases of abnormal uterine bleeding are associated with a secretory endometrium; some have been described as "irregular ripening" or "irregular shedding." In addition there are corpus luteum cysts which may be determined by physical examination and biopsy of the endometrium. It seems possible that testosterone propionate, if it does not harmfully depress the human ovary, may be of value in such cases by causing degeneration of the corpus luteum.

Summary. Testosterone propionate in daily doses of 10 mg over a period of 22 days did not prevent ovulation in the rabbit in response to human pregnancy urine. Under continued testosterone treatment degeneration of the newly-formed corpora lutea was accelerated.

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3 Makepiece, A. W., Weinstein, G. L., and Friedman, M. H., *Am. J. Physiol.*, 1937, **119**, 512.

4 Skowron, S., *Comp. rend. Soc. de biol.*, 1935, **119**, 431.

5 Courrier, R., and Gros, G., *Comp. rend. Soc. de biol.*, 1938, **127**, 921.

10587

Sulfapyridine*: Immunity to Reinfection with Type I Pneumococcus.†

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Whitby[1] found that mice infected with Type I pneumococcus and treated with sulfapyridine were immune, on recovery, to subsequent infection with this pneumococcus. Long[2] stated that he was unable to confirm this observation. The following experiments were performed in order to obtain more information on this disputed point.

One hundred twelve white mice were infected intraperitoneally, with approximately 100 lethal doses of a Type I pneumococcus, this quantity of organisms being contained in 10^{-7} cc of a 12-hour blood-broth culture. Twenty-four of these mice served as untreated controls. The remaining 88 received sulfapyridine; 20 mg of the drug, suspended in a 10% acacia solution, were administered orally 2, 8, 14, and 20 hours after infection and every 24 hours thereafter for 5 successive days. As Table I shows, 75 of the treated mice survived infection. At 7, 14, and 28 days after the initial infection, groups of these survivors were reinfected with 100 lethal doses of the same Type I pneumococcus. Groups of untreated mice served as controls for each of the above groups. Surviving mice were killed 7 days after reinfection. Cultures of heart-blood made at that time were uniformly negative.

The results summarized in Table I show that when mice were reinfected 7 and 14 days after the initial infection, 86% survived. When reinfected at the end of 28 days, only 6% survived.

Before concluding that the mice survived because of immunity to Type I pneumococcus, 2 other possibilities had to be considered: (1) that protection was due to the retention of small but therapeutically effective amount of sulfapyridine by the mouse, and (2) that sulfapyridine *per se* stimulated natural defensive mechanisms.

The first possibility seems unlikely, because experiments have

* Sulfapyridine was supplied by the Calco Chemical Company, Bound Brook, New Jersey, through the generosity of its Medical Director, Dr. D. A. Bryce.

† This work was supported in part by a grant from the Union Central Life Insurance Company, Cincinnati, Ohio.

1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

2 Long, P. H., *J. A. M. A.*, 1939, **112**, 538.

TABLE I.
Immunity of Sulfapyridine-treated Mice to Reinfection with Type I Pneumococcus.

Initial Infection-experiments					Reinfection-experiments				
			Survivors				Reinfection-days after initial infection	Survivors	
Group	No. of mice	Treatment	No.	%	Group	No. of mice		No.	%
I	88	Sulfapyridine	75	85	Survivors Group I	15	7	13	86
					Untreated controls	6	—	0	0
Untreated controls	24	None	0	0					
					Survivors Group I	14	14	12	86
					Untreated controls	10	—	0	0
					Survivors Group I	36	28	2	6
					Untreated controls	12	—	0	0

shown[3] that sulfapyridine administered to normal mice, as in the above experiments, was completely eliminated from blood and urine within 72 hours after the last dose of the drug. Yet mice were immune to reinfection as long as 8 days after the last dose of sulfapyridine.

The second possibility was eliminated by the following experiment. Twenty-five normal mice received 20 mg doses of sulfapyridine as described previously; 24 hours after the last dose of the drug, they were infected with 100 lethal doses of Type I pneumococcus. The administration of sulfapyridine did not increase the resistance of these animals for they were all dead within 36 hours, as were 12 untreated controls infected similarly.

These data justify the conclusion that mice that have recovered from infection with Type I pneumococcus, through treatment with sulfapyridine, are immune to reinfection for a limited time. Our experiments have confirmed Whitby's observation. Since Long has not presented his experimental data, it is impossible to explain the apparent discrepancy between his conclusion and that warranted by Whitby's results and our own. The discrepancy could be explained if one assumed that Long reinfected his mice later than 14 days after the initial infection. In this event our data would support his conclusion.

Summary. Mice recovering from a Type I pneumococcal infection, as a result of sulfapyridine therapy, are generally immune to reinfection for at least 14 days after the initial infection. This immunity is lost within 28 days.

10588 P

Effect of Artificially Induced Hyperpyrexia on Tooth Structure of the Rabbit.

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The increasing therapeutic importance of artificially induced fever raises the question of its effect on developing tooth structure. Damage to the enamel by infections accompanied by high fever during the

[3] Schmidt, L. H., and Hughes, H. B., results to be published.

period of tooth formation has been recognized clinically for many years. It is evidenced as a hypoplastic condition with superficial pits and grooves, often markedly disfiguring the anterior teeth. Detrimental effects in the dentin would be an internal dystrophy and would escape clinical notice but might well be expected under conditions which would affect the enamel.

Normal dentin is a homogeneously calcified tissue produced by the dental pulp. It is laid down around the periphery of the pulp in periodic increments as an organic matrix which is subsequently calcified to a bone-like consistency. Unlike bone, dentin once formed is not subject to further physiologic change to any marked degree, and hence it affords a permanent record of any variations in the calcification process. The dentin of the continuously growing teeth of the rabbit, therefore, affords a suitable medium for the recording of any effect which might be caused by artificially induced hyperpyrexia.

In the same series of rabbits used for observations on other tissues by the Departments of Medicine and Pathology and reported elsewhere,[1, 2, 3] histologic studies were made on thin ground sections cut longitudinally through the incisor teeth. Schour and Hoffman,[4] reporting on dentin deposition in growing mammalian teeth, state that it is laid down at the approximate rate of 16 micra in 24 hours. Their work included, among other animals, a series of rabbits. Thus, any periodic interference with calcification would be characterized by the presence in the dentin of alternate layers of normal and abnormal tissue, the latter coinciding with the periods of disturbance.

Sections of the incisor dentin of the rabbits, previously intermittently fevered at 106.5° to 108.5°F by both radiotherm and Kettering hypertherm and allowed definite intervening recovery periods, demonstrated linear striations characterized by the presence of numerous uncalcified spaces resulting from a lack of fusion of the individual calcification globules. These areas alternated with more homogeneously calcified tissue and corresponded in number with the number of fevering periods of the animal in question. The degree of interference with calcification, as judged by the size and frequency of the uncalcified spaces, was directly related to the intensity of the induced fever during the early periods of fevering. Subsequently the disturbance became less marked.

Poorly calcified tooth tissue, both enamel and dentin, offers less

1 v. Haam, E., and Frost, T. T., PROC. SOC. EXP. BIOL. AND MED., 1939,

2 Weaver, H. M., in press.

3 Hargraves, M. M., Doan, C. A., and Kester, L., in press.

4 Schour, I., and Hoffman, M. M., *J. D. Res.*, 1935, **15**, 161.

resistance to the progress of dental caries than does a well calcified structure. In addition, the presence of interglobular spaces in the dentin increases the pain incident to the preparation of cavities for dental restorations. Thus the importance of efforts to reduce or eliminate this undesirable effect of artificially induced fever on tooth structure becomes quite apparent.

While the use of sodium fluoride injections and vital staining with alizarine have already provided means of inquiring into some of the biological processes associated with dental calcifications, controlled artificial fever would seem to offer another and somewhat different approach to the study of such physiological phenomena.

Further work, using the albino rat as the subject of artificially induced hyperpyrexia, is being carried on in order to observe the effects over a wider range and duration of temperatures, and in an attempt to lessen the undesirable effects by a preliminary increase of the available supply of materials concerned in the calcification process.

10589 P

Pathogenesis of Hemorrhage in Artificially Induced Fever.

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Every therapeutic agent of value has its potential dangers and hyperpyrexia is no exception to this general rule. Two of the most constant pathologic findings following induced fever in experimental animals and in human subjects are hemorrhage and acute parenchymatous degeneration of the liver. Hartman[1] has pointed out the similarity of these pathologic changes to those encountered following prolonged mild asphyxia, and has demonstrated that anoxia is a common accompaniment of artificially induced fever.

The mechanism underlying the hemorrhage following artificially induced fever has never been entirely satisfactorily explained. In the present study selected cellular and humoral factors important in the complex phenomenon of blood coagulation have been determined under experimental conditions in rabbits and during fever therapy in human patients. Total platelet counts have been correlated with

[1] Hartman, F. W., *J. Am. Med. Assn.*, 1937, **109**, 2116.

qualitative and quantitative studies of the megakaryocytes in serial marrow punctures; quantitative prothrombin and fibrinogen determinations have been followed by histopathologic studies of the liver. Fever was induced in the experimental animals with the radiotherm and in human subjects with the Kettering hypertherm.

Marked differences in the susceptibility of these several coagulation factors to fever were noted from individual to individual. During and following artificially induced fever in rabbits there was a decrease in total platelets in all instances, the lowest determinations being one-fourth to one-third of the pre-fever control values. More or less extensive megakaryocytic damage was apparent during the period of low platelet values and a prompt and rapid regeneration of new megakaryocytes always preceded the return of the circulating platelets to normal levels. A quantitative decrease in prothrombin and fibrinogen occurred in those animals in which liver damage was later found, and where no hepatic damage could be demonstrated no disturbance in prothrombin had been recorded.

Comparable observations were made in young adult patients, both male and female, normal except for some manifestation of gonorrheal infection. The efficiency and integrity of the liver were determined from time to time by the appropriate liver function tests and by variations in the icterus index. There was a pathologic retention of bromsulphalein dye in every subject immediately following induced fever together with a greater or lesser degree of chemical and clinical jaundice (highest icterus index was 70 units). The most marked decrease in prothrombin occurred during the 24-hour period following the fever treatment. Some decrease in prothrombin occurred in every fever patient studied, though in only one individual did the determination fall below the critical level of 35% of normal. In this individual who received 10 hours of hypertherm fever above 105°F without previous glucose fortification, the prothrombin reached a low point of 11% of normal and hematemesis occurred during that time. Quantitative fibrinogen studies in 2 subjects showed no variation from normal values. The circulating blood platelets were affected in all subjects, the maximum decrease being approximately one-sixth to one-fourth the control values, sometimes persisting for 48 to 72 hours post-fever. Epistaxis occurred in one individual on the day following fever therapy during this period of thrombocytopenia. A much slower tendency for megakaryocytic regeneration was noted in the human than in the rabbits' marrow.

On the basis of these studies, the sequence of events in the pathogenesis of hemorrhage in artificially induced fever may be reconstructed as follows: with the rise in temperature anoxia develops, a

rapid depletion of the liver glycogen occurs and definite histologic evidence of hepatic cell necrosis appears. The decrease in prothrombin, with or without a decrease in fibrinogen, may be correlated with the extent of liver damage. Any injury or destruction to megakaryocytes is reflected by a prompt proportionate decrease in the circulating blood platelets. Concomitant damage to the endothelial cells directly has not been demonstrated.

Whether hemorrhage will remain potential or become an actual fact in any given instance must depend upon the relative degree and interaction of the impairment which each of these 3 important coagulation factors suffers during a period of fever. The assurance of adequate glycogen storage in the liver should always be prerequisite to fever therapy, and a careful appraisal should be made of the megakaryocytic reserves in the bone marrow.

10590

Effect of Pectin Supplements on Avitaminosis A in Rats.*

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It is generally recognized that vitamin A is essential in preventing xerophthalmia and keratinization of the mucous epithelium of the body. This substance is also thought to play an important rôle in the synthesis of mucin by the body because a dehydration of the mucous membrane occurs as a result of avitaminosis A. Manville[1, 2] has suggested that galacturonic acid plays a rôle in mucin formation. In view of the fact that the pectin molecule is composed of 8 molecules of galacturonic acid, it was thought that ingested pectin when fed in a vitamin A-free diet might retard the characteristic keratinization of the mucous epithelium. The object of this investigation was to determine whether or not pectin is effective in preventing or

* Contribution No. 317, Massachusetts Agricultural Experiment Station. Abstracted from A. Kobren, Thesis for Master of Science degree, Massachusetts State College, 1938.

1 Manville, I. A., Address delivered at 41st annual convention International Apple Assn., Boston, Mass., 1936.

2 Manville, I. A., *Science*, 1937, **65**, 44.

delaying the development of the typical pathological symptoms which occur in avitaminosis A. The citrus pectin was "pure, 160 grade" obtained from the California Citrus Products Corporation, Ontario, California. It was free from vitamin A.

Four groups of female white rats were used in the experiment, the rats within each group being litter mates.

In Group 1, 6 rats were placed on a vitamin A-free diet, 6 rats on a vitamin A-free diet with the addition of 6% pectin, and 2 rats on a control diet containing an adequate amount of vitamin A.

In Group 2, 5 rats were placed on a vitamin A-free diet, with the addition of 3% pectin, 5 rats on a vitamin A-free diet, and 2 rats on the control diet.

In Group 3, four rats were placed on a vitamin A-free plus 12% pectin diet, and 2 rats were placed on the control diet. The 12% pectin diet was employed in order to observe the preventive action of pectin in regard to macroscopic manifestations of avitaminosis A. The diets employed were modifications of the Sherman and Munsell vitamin A-free diet.

The animals were observed carefully during the experiment, care being taken to keep the food and water fresh and amply supplied. The weights and macroscopic changes were recorded every other day, and upon later manifestations of avitaminosis A, daily observations were recorded.

The first part of the investigation consisted in making vaginal smears to detect the earliest possible indication of avitaminosis A. The method followed was that described by Mason and Ellison.[3] At the onset of xerophthalmia in litter mates, the particular litter was killed and histological sections were made of the following tissues: eyelids, nares, vagina, liver, oesophagus, small intestine, and kidney.

The use of pectin as a supplement in a vitamin A-free diet did not delay the onset of xerophthalmia nor did it act in a curative capacity relative to this condition. In the liver, small intestine, kidney, and oesophagus there was no marked changes in avitaminosis A as compared to similar tissues from normal animals. In the vagina, nares, and eyelids there were marked changes in the mucosae and submucous coat. The characteristic manifestations of avitaminosis A occurred in the rats on the vitamin A-free diet. However, in the rats receiving pectin these changes were not so marked.

Conclusion. Pectin appeared to be a beneficial supplement to a diet deficient in vitamin A, only insofar as pathological changes due to avitaminosis A in the vagina, nares, and eyelids are concerned.

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[3] Mason, K. E., and Ellison, E. T., *J. Nutr.*, 1935, **9**, 735.

10591

Failure of Streptococcal Antibodies to Influence Chemotaxis of Leukocytes.*

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Is chemotaxis of leukocytes a specific immunological reaction; is it, like phagocytosis and agglutination, enhanced by the presence of bacteriotropins, or, on the contrary, does chemotaxis occur just as well in the absence of specific antibodies, in the normal as in the immunized animal? It is well known that polymorphonuclear leukocytes display positive chemotaxis to most or all kinds of bacteria, and advance toward them by amœboid movement. However, it is not known whether chemotaxis is aided by the presence of specific antibodies.

To obtain information on this question, the chemotactic response of rabbits' polymorphonuclear leukocytes to streptococci was observed *in vitro,* and measurements were made to find whether the reaction is increased by the addition of streptococcal antibodies.

The method used in these experiments has been described in detail elsewhere.[1] Essentially it consists in observing with the microscope the direction of locomotion of polymorphonuclear leukocytes in proximity to a clump of bacteria. The bacteria, grown in liquid medium, are washed twice in distilled water, and a small loopful of the concentrated bacterial suspension is placed on a glass slide. After drying, the bacteria form a flat, round or oval mass about 1 mm in diameter. Leukocytes are obtained from the peritoneal cavity of the rabbit by injecting 150 cc of physiological saline and removing it 4 hours later; it then contains great numbers of polymorphonuclears. These are concentrated by gentle centrifugation and are then mixed with plasma obtained from the heart blood of the same animal. No anticoagulant is used. The suspension is made in such a way that each high-power field of the microscope contains from 10 to 25 leukocytes. A drop of the plasma-leukocyte suspension, while still liquid, is placed on a coverslip and superimposed on the glass slide, with the bacterial clump in the center of the preparation. This is sealed to prevent evaporation and is observed with the microscope at

* This investigation was aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1 McCutcheon, M., Coman, D. R., and Dixon, H. M., *Arch. Path.,* 1939, **27**, 61.

37.5°C. The image of a microscopical field containing a portion of the bacterial clump and a number of leukocytes is projected on paper by means of a drawing ocular, and the position of each leukocyte is recorded at intervals of a minute for 10 minutes. The distance of each leukocyte from the bacterial clump at the first observation is measured, and these distances are added. Similarly the sum of the distances at the last observation is found. The difference between these sums is divided by the total minutes of observation for all the leukocytes, thus giving the value of chemotropism in microns per minute for each microscopical field. This value has a positive sign if the cells taken collectively move toward the bacteria, a negative sign, if away. In strong positive chemotropism, a value of the order of +10 microns per minute is expected, while in strong negative chemotropism an equally high negative value is obtained.

Since the object of these experiments was to find out whether acquired antibodies increase the attraction of leukocytes to bacteria, a strain of organisms was selected which did not attract leukocytes strongly in the absence of such antibodies; in this way any increase in attraction due to antibodies would be more easily detectable. A suitable organism was obtained from the Department of Bacteriology, *Streptococcus hemolyticus* (strain 1048). It had already been found[1] that this organism attracts leukocytes for only a few minutes under the present conditions. We now planned experiments to show whether attraction would be increased in intensity or prolonged in time by the presence of agglutinating and phagocytosis-promoting substances.

In the first series of experiments, bacteria were sensitized with immune rabbit serum; leukocytes and plasma were obtained from normal animals. Sensitization of streptococci of this strain greatly increases phagocytosis.[2] Our experiments gave an opportunity to observe whether conditions that increase phagocytosis also increase chemotaxis.

Lyophilized serum was prepared by Dr. David Lackman of the Department of Bacteriology, and was used in the dilution of 1:256, which was the limiting dilution of maximal agglutination. Streptococci, usually from 18-hour cultures in neopeptone broth, were sensitized from 30 to 90 minutes at 37°C. Then they were washed with distilled water, and a loopful placed on a glass slide, as already described. As control, a loopful of bacteria treated with normal rabbit serum was placed on the same slide about one cm from the

[2] Mudd, S., Czarnetzky, E. J., Lackman, D., and Pettit, H., *J. Immunol.*, 1938, **34**, 117.

sensitized organisms. Thus the chemotactic effects of sensitized and unsensitized streptococci could be compared in the same preparation.

Ten-minute records were then made of the movements of leukocytes near the sensitized and unsensitized bacteria in turn. These observations were repeated after half an hour. During the first period of observation, chemotaxis was moderately strong toward both sensitized and unsensitized bacteria. In 14 preparations the mean value of chemotaxis was, with sensitized streptococci, +7.9 microns per minute; with unsensitized bacteria, +7.4 microns per minute. The difference is obviously not significant.

During the second period of observation, in 10 preparations, chemotaxis brought about by sensitized bacteria fell to +3.9 microns per minute; with bacteria treated with normal serum a value was obtained of +2.1 microns per minute. The difference with its standard error is 1.8 ± 1.11 and is not statistically significant. It is concluded that under these conditions, sensitization of bacteria by specific antibodies did not increase the intensity of chemotaxis nor prolong the duration of the reaction.

The second series of experiments was designed to reproduce more closely conditions in actual infections, that is, the antibodies were present in the cell-plasma suspension surrounding the bacteria. Unsensitized bacteria were used, and cells and plasma were obtained from rabbits immunized by repeated inoculation with living hemolytic streptococci (strain 1048). The pooled serum of the 6 rabbits used was found by Dr. Lackman to have an agglutinating titer of 1:4096. In control experiments, cells and plasma were obtained from normal rabbits. The sera of none of the normal rabbits, tested separately, had agglutinating power. The bacteria in these experiments were from relatively old cultures (several days), since it appeared that bacteria from an old culture exerted less attraction than younger organisms, and therefore gave better opportunity to detect any increase in chemotaxis brought about by antibodies.

Nineteen pairs of preparations were observed. During the first period of observation, with cells and plasma of immunized animals the mean value of chemotaxis was +3.7 microns per minute; in preparations made with cells and plasma of normal animals, +4.5 microns per minute. The difference with its standard error is 0.8 ± 0.83, and is not significant. During the second period of observation, chemotaxis ceased in both types of preparation, the leukocytes appearing to move at random. In 15 preparations with cells and plasma of immunized animals, the mean value of chemotaxis was +0.8 microns per minute, in preparations made from normal animals, —0.9 microns per minute. The difference is not sig-

nificant. It is concluded that under the conditions of these experiments the presence of agglutinating and phagocytosis-promoting antibodies in the plasma fails to increase or prolong chemotaxis.

Thus no evidence has been obtained from these experiments favoring the view that chemotaxis is dependent upon or is increased by the presence of agglutinating and phagocytosis-promoting antibodies. It seems rather that chemotaxis is brought about primarily by substances produced by the organisms,[3] and that such substances are given off by all kinds of bacteria.[4] If this is true, chemotaxis is not a specific immune reaction as are, in part, phagocytosis and agglutination, but a non-specific response. It appears that antibodies play their part only after the cell has been attracted to the bacteria; then, by facilitating the spread of cell on particle,[5] antibodies aid in bringing about phagocytosis.

Summary. Sensitization of a strain of hemolytic streptococcus with rabbit antiserum did not increase the chemotactic attraction of these bacteria for rabbit polymorphonuclear leukocytes *in vitro*. Also the chemotactic response to hemolytic streptococci was no greater when leukocytes and plasma were obtained from immunized rabbits than when they were obtained from normal animals. Under these conditions, chemotaxis, unlike phagocytosis and agglutination, is not increased by specific antibodies, but appears to be rather a non-specific response of leukocytes toward microörganisms.

10592

Studies on *H. pertussis*.* I. Liberation by Sonic Vibration of a Soluble Component That Absorbs Phase I Agglutinins.

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(Introduced by D. W. Bronk.)

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The antibacterial action of protective antisera has been shown to involve combination with antigens present on the bacterial surface.

3 Dixon, H. M., and McCutcheon, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 378.

4 McCutcheon, M., and Dixon, H. M., *Arch. Path.*, 1936, **21**, 749.

5 Mudd, S., McCutcheon, M., and Lucke, B., *Physiol. Rev.*, 1934, **14**, 210.

* This work has been aided by a grant from the United States Public Health Service.

The preparation from non-flagellated organisms of a soluble antigen detectable by such surface-reactions as agglutination or phagocytosis is therefore of cardinal importance in relation to antibacterial immunity. With this as a guiding principle we have tested the commercial preparation of Krueger's Ball-mill "Endoantigen" of *H. pertussis*[1] but have found it, as purchased, not to contain detectable quantities of agglutinin-absorbing components. In view of this and the apparent lack of value of Endoantigen in the prophylaxis of whooping cough[2, 3] we have considered it worthwhile to apply the sonic method of extraction[4] to suspensions of *H. pertussis* in Phase I with the hope of obtaining a soluble agglutinogen. Other available preparations of soluble antigens of *H. pertussis* have not been so extensively studied as Endoantigen and this report includes results obtained with others currently used in clinical practice.

Phase I organisms freshly regenerated from Cryochem-dried form[5] were grown in Roux flasks on Bordet-Gengou medium containing 20% of fresh defibrinated horse-blood. Not more than 10 culture-generations were used following the opening of a container of dried organisms and the final generation was never more than a month removed from the dry form. The organisms were harvested by scraping the medium after 72 hours of incubation at 37°C and were suspended in saline. Less blood was removed with the organisms by scraping than by washing. Such suspensions in concentration of approximately 1000 billion organisms per milliliter were subjected to sonic disintegration for one hour in apparatus similar to that previously described.[6] The suspensions were then centrifuged at high speed and the opalescent supernate was diluted with sterile distilled water to 10 times its volume. The diluted extract was passed through a Seitz bacterial filter by suction and the clear filtrate was then concentrated. This was done by drying from the frozen state in the Cryochem apparatus[7] and restoring with sterile distilled water to the volume of the original supernatant solution. Small amounts of material not readily soluble were removed by centrifugation.

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1 Kreuger, A. P., *J. Infect. Dis.*, 1933, **53**, 237.

2 Roundtable Conference on Vaccine Prophylaxis of Whooping Cough, *J. Ped.*, 1938, **13**, 277-300.

3 Singer-Brooks, Charlotte H., *J. Pediat.*, 1939, **14**, 25.

4 Chambers, L. A., and Flosdorf, E. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 631.

5 Flosdorf, E. W., and Kimball, A. C., to be published.

6 Chambers, L. A., and Flosdorf, E. W., *loc. cit.*

7 Flosdorf, Earl W., and Mudd, S., *J. Immunol.*, 1938, **34**, 469.

Antiserum against Phase I organisms of a titer of 1:25,000 was then absorbed with this reconstituted filtrate; .05 ml of undiluted serum was incubated for 30 minutes at 37° with 0.45 ml of the filtrate and then for 4 hours at 4 to 8°C. The precipitate was removed by centrifugation and 0.05 ml of the supernate was incubated 30 minutes at 37° with another 0.45 ml portion of the sonic filtrate and overnight in the refrigerator. The precipitate was removed and 0.3 ml of the supernate was incubated 30 minutes with 0.9 ml of the filtrate and overnight in the refrigerator. At this point there remained only a slight trace of precipitate which was removed. The supernate accordingly represents a serum-dilution of 1:400.

The absorbed serum was tested by agglutination in serial dilutions from 1:400 to 1:25,600 in steps of 1:2; 0.5 ml of each dilution was incubated 30 minutes at 37° and overnight in the refrigerator with 0.5 ml of a two billion per ml suspension of living Phase I organisms. In each case normal serum was exposed to the same absorbing antigen under identical conditions in order to detect possible non-specific agglutination. Also the immune serum was treated identically using saline in place of the absorbing antigen. The organisms used for production of the immune rabbit serum were grown on medium containing horse blood. Therefore, in some cases the test-antigen was grown on medium containing sheep blood in order to evaluate the remote possibility that horse-blood constituents in the sonic filtrate could cause absorption.

The serum absorbed with sonic filtrate showed no agglutinating power even at 1:400 (1:800 final dilution) when tested with the homologous Phase I organisms as antigen. (Table I). However, 6 absorptions using undiluted serum absorbed with equal volumes of sonic filtrate, rather than the lower ratio of serum to absorbent as described above, resulted in only partial absorption of agglutinins, which indicates that the antibody-content of such high-titer serum cannot be removed to completion unless the serum is diluted. The nitrogen-content of the sonic filtrate as used in the absorption was only 0.7 to 1.6 mg per ml. This low concentration of the sonic filtrate suggests why the technic of diluting the serum as described was necessary. The control experiments described above in every case validate the specificity of absorption by the sonic filtrate.

Using even the higher ratio of antigen-volume to serum-volume, saline washings of the whole living organisms, Kreuger's endo-antigen, a commercial preparation known at Topagen† and another

† Samples of "Topagen" with and without a mercurial preservative were kindly furnished by Dr. John Reichel and Mr. C. Roos of Sharp and Dohme.

TABLE I.
Typical Agglutinin-titrations.*

Absorbing antigen	Mg nitrogen per ml	Precipitation during absorption			Final antiserum-dilutions						
		First	Second	Third	1/800	1/1600	1/3200	1/6400	1/12,800	1/25,600	1/51,200
Unabsorbed	—	—	—	—	4	4	3	2	2	1	1
Sonic filtrate*	0.70	2	1	tr	0	0	0	0	0	0	0
Saline washings of whole living organisms	—	tr	tr	0	4	4	4	3	tr	tr	tr
Endo-antigen	0.11	tr	tr	tr	4	4	3	2	1	1	1
Topagen	0.54	1	tr	tr	4	4	3	3	2	2	tr
"Detoxified" antigen	3.14	tr	tr	tr	4	4	4	3	2	2	1

*Similar results have been obtained in each of the 12 experiments in which absorption was carried out with sonic filtrate using the lower ratio of serum to absorbent as described. Five different batches of sonic filtrate were used in the course of these experiments. In the case of the other absorbents, the experiments were carried o ut at least 3 times and except in the case of "detoxified" antigen at least 2 different preparations or containers were used.

known as Detoxified Pertussis antigen‡ effected no detectable removal of agglutinins (Table I). This finding with respect to the last 2 preparations cannot be interpreted *necessarily* in terms of their possible effectiveness. The mechanism of action postulated for the reported effectiveness of Topagen[8] is not immunization against the antigens at the surface of the organism. Likewise the last preparation is being offered as a possible control of whooping cough through a toxin of *H. pertussis*.

The results of precipitin-testing have not been included. This method of assay, like complement fixation, does not distinguish components of the surface of the cell from other cellular or metabolic and medium-components.

In view of the fact that intense sonic treatment produces chemical changes in certain systems and not in others[9-12] the sonic filtrates were exposed to further sonic treatment for an hour. No detectable loss in agglutinin-absorbing capacity was observed.

The agglutinin-absorbing material present in the sonic filtrate may be either a whole antigen or a hapten. Further work is in progress to determine this by experiments with animals and to isolate and identify the component chemically. The authors wish to express their appreciation to Dr. Stuart Mudd for his helpful suggestions during the course of this work.

Summary. Filtered extracts of *H. pertussis* obtained by sonic disintegration of Phase I organisms have been found to absorb to completion the agglutinins from homologous rabbit antiserum of high titer. The soluble preparations now available and known as Endoantigen, Topagen and Detoxified Pertussis antigen were found to be devoid of such activity within the limits of sensitivity of the method used.

‡ Manufactured by Lederle Laboratories, Pearl River, New York. (Purchased on the market.)

8 Gold, H., *J. Ped.*, 1937, **10**, 641.

9 Flosdorf, E. W., and Chambers, L. A., *J. Am. Chem. Soc.*, 1933, **55**, 3051, 1934, **56**, 2795.

10 Flosdorf, E. W., Chambers, L. A., and Malisoff, *J. Am. Chem. Soc.*, 1936, **58**, 1069.

11 Chambers, L. A., and Flosdorf, E. W., *J. Biol. Chem.*, 1936, **114**, 75.

12 Flosdorf, E. W., and Chambers, L. A., *J. Immunol.*, 1935, **28**, 297.

10593 P

Melanin: a Natural Reversible Oxidation-Reduction System and Indicator.*

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The phenol-indophenol dyes induce a pallor in amphibian larvæ[1, 2] by inhibiting the enzyme tyrosinase.[3] During experiments, the results of which indicate that the enzyme tyrosinase is regulated by oxidation-reduction potentials, it was desirable to reduce certain oxidation-reduction indicator-dyes present in solutions of melanin. It was found that sodium hydrosulfite not only decolorized the dyes but also changed black melanin to a light brown or tan color. The same thing happened in melanin-solutions containing no dyes, and the pigment could be brought back to the black oxidized state by the addition of potassium ferricyanide. This was repeated in the same solution several times, so it was concluded that melanin is a natural reversible oxidation-reduction system.

The degree of color-change is indicated by the change in light-absorption determined by means of a photo-electric colorimeter. An absorption value of 0.0 was arbitrarily assigned to water. A solution of oxidized melanin absorbed 70% of the light. When reduced with sodium hydrosulfite, it absorbed only 25% of the light. On reoxidation, it again absorbed 70% of the light. Melanin produced by mealworm-tyrosinase, potato-tyrosinase, and autoöxidation of dihydroxyphenylalanine all show this same reversibility. Natural melanin appears to respond more sluggishly and to a lesser degree, but this may only be due to the necessarily low concentrations obtainable and the contaminations present in solutions of natural melanin.

The reduced form of melanin decolorizes the dyes in the series of redox indicators above and including toluylene blue. The oxidized form of melanin oxidizes the reduced indigo disulphonate, methylene blue, and thionine. Melanin must, therefore, be regarded as a natural redox system comparable to glutathione, riboflavin, and cevitamic acid. It has a relatively high potential and since it is highly colored and widely distributed, its very presence and color may become a valuable intracellular redox indicator.

* Aided by a grant from the American Association for the Advancement of Science.

1 Lewis, M. R., *J. Exp. Zool.*, 1932, **64**, 57.

2 Figge, F. H. J., *Ibid.*, 1938, **78**, 471.

3 Figge, F. H. J., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 569.

10594

Masculinization of the Female Rat by Gonadotropic Extracts.

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The original purpose of this investigation was to repeat an experiment of Selye and Collip[1] in which continuous vaginal estrus was obtained in immature rats treated with an anterior pituitary-like extract. Injections were started on the sixth day of age and continued until the thirtieth day but the dose employed was not stated. As a preliminary test, 8 six-day-old female rats were treated daily with 1 R.U. of anterior pituitary-like substance (Antuitrin-S). Vaginal patency occurred from the seventeenth to the nineteenth day of age but continuous estrus was not demonstrated by daily vaginal smears. When the dose was increased to 2 R.U. of Antuitrin-S daily, vaginal patency occurred from the fourteenth to the twentieth day but only 2 of 17 rats had continuous estrous smears. Daily doses of 5 R.U. induced vaginal patency from the sixteenth to the twentieth day but only 2 of 7 rats had smears suggestive of continuous estrus. Ten rat units daily resulted in continuous estrus in 7 of 8 rats treated. In this group the smears were continuously estrous from the fifteenth and seventeenth days, when vaginal patency occurred, until the termination of the experiment on the thirtieth day. The present report is concerned with a masculinizing effect that became more apparent as the trial doses were increased.

The animals used in this study were female rats selected at 6 days of age and made up in groups of 8 to standardize litter size and facilitate treatment. To date 24 of these selected litters have been treated and several others have been utilized as controls. Daily injections of gonadotropic substances were given from the sixth until the thirtieth day of age.

Definite masculinization as evidenced by hypertrophy of the clitoris occurred in 6 of 8 litters which received 2 R.U. of Antuitrin-S daily or its equivalent of Antophysin or A.P.L. The hypertrophy of the clitoris was evident after 10 days and increased with further treatment. The prepuce developed so that it could be drawn back to expose the clitoris. At 30 days of age the preputial glands were at least twice as large as those of the controls and an abundance of waxy

[1] Selye, H., and Collip, J. B., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 647.

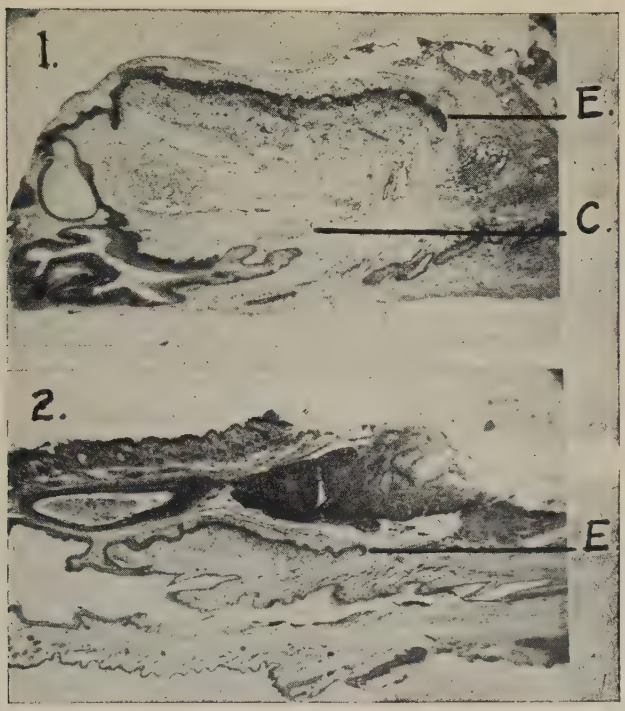

FIG. 1.
Median longitudinal section through the clitoris of a female rat 33 days of age treated with Antuitrin-S, 2 R.U. daily, from the 6th day of age. E. Epithelium of glans clitoridis. C. Cartilage.

FIG. 2.
Median longitudinal section through the clitoris and prepuce of a female rat 31 days of age, normal untreated control. E. Epithelium of glans clitoridis.

secretion was easily expressed. The glans clitoridis resembled a small glans penis and the horny spicules covering the glans were nearly as prominent as those in the male. The penile hypertrophy was limited to the glans and the cartilage anlage of the os priapi developed in the treated rats (Fig. 1). Ten R.U. of Antuitrin-S daily produced an even greater development of the clitoris.

To determine whether this masculinizing effect was peculiar to anterior pituitary-like preparations, 2 litters were treated with pregnant mare's serum (Gonadogen), 0.5 R.U. daily. The vaginæ were patent by the fourteenth day of age and the development of the clitoris and preputial glands was comparable to that of rats treated with 10 R.U. of Antuitrin-S. When 2 litters were treated with a pituitary extract (Prephysin), 5 R.U. daily, vaginal patency occurred from the sixteenth to the eighteenth day of age but there was no appreciable enlargement of the clitoris or preputial glands.

For comparison 2 litters were treated with estrone (Amniotin) and 2 with testosterone (Perandren). Ten international units of estrone were given on alternate days until the fourteenth day of age, when this dose was increased to 125 international units. Vaginal patency occurred on the sixteenth day and the vaginal smears remained consistently estrous during the period of treatment. There was no enlargement of the clitoris. Two litters were given 0.3 mg testosterone acetate on alternate days. The vaginæ were all patent by the fourteenth day of age but the smears were consistently diestrous. The development of the clitoris and preputial glands was greater in rats treated with testosterone than in those treated with Antuitrin-S or Gonadogen. The masculinization with testosterone was entirely comparable to that reported by Greene, Burrill and Ivy.[2]

Treatment with anterior pituitary-like extracts continued beyond 30 days of age did not cause any further increase in the size of the phallus. Cessation of treatment at 30 days was followed by some regression of the induced masculinization. The development of the clitoris and preputial glands was greatest in those rats in which the dosage was sufficient to induce continuous estrus. Apparently the infantile rat responds to gonadotropic treatment in a bisexual manner by producing effective amounts of both estrogenic and androgenic substances.

The results reported here are anticipated by Greene, Burrill and Ivy.[3] They may be comparable to those of Papanicolaou and Falk[4] and Guyenot and Naville-Trolliet[5] in which they induced masculinization of the female guinea pig by pregnancy urine and anterior pituitary extracts. The ovary was necessary to mediate the effect since pregnancy urine extract was not effective in ovariectomized guinea pigs. Domm[6] reported masculinization of the newly hatched chick by treatment with an anterior pituitary extract. The ovarian response in these young chicks was characterized by a marked hypertrophy of the medullary portion of the ovary and an absence of any follicular stimulation. It appeared that the medullary portion of the chick ovary was the probable source of androgenic hormone. A recent paper[3] suggests that the androgen produced by ovaries may be

2 Greene, R. R., Burrill, M. W., and Ivy, A. C., *Am. J. Obst. and Gynec.*, 1938, **36**, 1038.

3 Greene, R. R., Burrill, M. W., and Ivy, A. C., *Endocrinology*, 1939, **24**, 351.

4 Papanicolaou, G. N., and Falk, E. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 750.

5 Guyenot, E., and Naville-Trolliet, I., *Revue Suisse de Zoologie*, 1936, **43**, 415.

6 Domm, L. V., *Cold Spring Harbor Symposia on Quantitative Biology*, 1937, **5**, 241.

progesterone. In agreement with earlier reports there was no follicular maturation or luteinization in the earlier stages of treatment in our rats.

Summary. Gonadotropic extracts of human pregnancy urine or pregnant mare's serum cause masculinization of female rats if treatment is started at 6 days of age and continued until the thirtieth day. The hypertrophy of the clitoris, prepuce and preputial glands is quite comparable to that induced by a similar course of treatment with testosterone. A gonadotropic pituitary extract did not cause any masculinization.

10595

Absorption of Glucose from the Stomach of the Dog.

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It is surprising that on so simple a subject as the fate of glucose in the stomach the published opinion is not of one accord.[1–13] Verzar and McDougall[14] state that "there is practically no absorption of carbohydrates in the stomach."

London and Tschekunow[9] in experiments on dogs with gastric fistulæ concluded that glucose was not absorbed during approximately a one-hour period. McLeod and his associates[10] reported similar observations in the rat when the pylorus was ligated.

More recently Maddock, Trimble and Carey[11] reported data from experiments on dogs from which they concluded that there was no

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2 Von Ansep, B., *Arch. Anat. und Physiol.*, 1881, page 504.

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5 Brandl, J., *Z. f. Biol.*, 1892, **11**, 277.

6 Edkins, N., *J. Physiol.*, 1928, **65**, 381.

7 Freund, I., and Steinhardt, P., *Deutsch. med. Woch.*, 1931, **57**, 1815.

8 Holtz, F., and Schreiber, E., *Biochem. Z.*, 1930, **1**, 224.

9 London, E. S., and Tschekunow, J. S., *Z. physiol. Chem.*, 1913, 313.

10 MacLeod, J. J. R., Magee, H. E., and Purves, C. B., *J. Physiol.*, 1930, **70**, 404.

11 Maddock, S. J., Trimble, H. C., and Carey, B. W., *J. Biol. Chem.*, 1933, **103**, 285.

12 Maddock, S. J., *J. Lab. and Clin. Med.*, 1932, **17**, 369.

13 Shay, H., Gershon-Cohen, J., and Fels, S. S., *Ann. Int. Med.*, 1938, **11**, 1563.

14 Verzar, F., and McDougall, E. J., *Absorption from the Intestine*, Longmans, Green and Co., London, Eng., 1936.

absorption of glucose from the stomach and no change in the blood sugar level of either the gastric or peripheral venous blood after the ingestion of various concentrations of glucose. In one series of experiments the pylorus was ligated and solutions of glucose, varying in concentrations from 5 to 46%, were introduced into the stomach and allowed to remain for a period of from 1 to 2 hours. Following this technic a mean recovery of 99.3% of the sugar introduced was obtained and there was no appreciable rise in the blood sugar level. They interpreted their results as demonstrating that glucose was not absorbed from the stomach in significant quantities.

In a series of experiments which we have conducted we have been unable to confirm the findings of Maddock, Trimble and Carey.[11]

In these experiments dogs varying in weight from 5 to 15 kilos and fasted for 24 hours were used. Sodium amytal anesthesia was used because it has less effect on the blood sugar level than other commonly used anesthetics, although we have found a slight but definite increase in the blood sugar level during a one-hour period of anesthesia. After the animal was placed on the table a sample of blood was taken from the femoral artery for determination of its sugar content. The determinations were made by the Folin-Wu[15] method. The animal was then injected with 50 mg of sodium amytal per kilo of body weight intraperitoneally. Samples of blood were removed at 30 and 60 minutes after the administration of the sodium amytal. One hour after anesthetization the abdomen was opened and the pylorus clamped with a heavy intestinal clamp, care being taken not to injure the blood supply to the stomach, either along the greater or lesser curvature. An incision was then made in the neck and the esophagus exposed and opened. A stomach tube was passed into the stomach through the esophageal opening and a ligature passed around the esophagus, fixing the position of the tube. The esophagus was also tied to the tube just below the diaphragm. The glucose solution was then introduced through a stomach tube, the tube washed free of glucose with water, and then clamped. In all the animals blood was removed every 15 minutes for determination of the sugar and the experiment terminated one hour after the introduction of the glucose.

Bacto-dextrose (Difco) was used for every experiment, the concentrations varying from 5.9 to 47.1%. The amount of fluid introduced varied from 200 to 300 cc. The sugar determinations on the introduced and recovered solutions were made by the Benedict method.[16]

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15 Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

16 Benedict, S. R., *J. Biol. Chem.*, 1911, **9**, 57.

TABLE I.

Changes in Concentration and Volume of Glucose After Introduction in the Stomach with the Change in Blood Sugar.

Dog	Wt,	Amt of glucose in			Amt of glucose out, 1 hr			Loss	Blood sugar	
									1 hr after Amytal,	1 hr after Glucose,
No.	kilo	cc	%	g	cc	%	g	g	mg %	mg %
1	12.0	200	47.1	94.2	240	29.5	70.8	23.4	83	250
2	16.0	200	45.0	90.0	240	32.8	78.7	11.3	92	122
3	5.6	220	44.6	98.1	240	31.3	75.1	23.0	84	202
4	15.0	200	43.3	86.6	270	27.7	74.8	11.8	—	—
5	6.3	220	41.7	91.7	220	33.6	73.9	17.8	104	212
6	14.0	225	40.5	91.1	285	29.2	83.2	7.9	100	133
7	6.8	220	40.4	88.9	250	32.7	81.8	7.1	93	133
8	13.3	220	15.0	33.0	250	12.6	31.5	1.5	83	86
9	9.6	300	14.9	44.7	305	13.6	41.5	3.2	121	131
10	14.5	230	6.8	15.6	275	5.5	15.1	0.5	90	86
11	15.0	200	6.1	12.1	215	5.5	11.8	0.3	71	86
12	14.8	200	5.9	11.8	210	5.5	11.6	0.2	93	104

At the completion of each experiment the stomach and esophagus were excised, the contents carefully emptied and the mucous membrane carefully washed. The washings were added to the contents which had been removed.

The final dilutions before determination by the Benedict method[16] were such that the titer of the recovered solutions approximated that of the original solutions. No allowance was made for any possible loss of sugar by bacterial action since it had previously been demonstrated by Cori[17] and confirmed in this laboratory that this action is insignificant.

In 7 dogs the concentrations of glucose introduced into the stomach varied from 40.4 to 47.1%. The increase in volume of the recovered contents did not account completely for the reduction in the concentrations of the recovered solutions. There was constantly a loss of glucose, the minimum and maximum amounts being 7.1 and 23.4 g respectively (Table I).

In 2 dogs solutions of approximately 15% glucose were used. In each instance evidence was obtained that a small amount of glucose was absorbed.

Approximately isotonic solutions were used in 3 dogs. In these experiments the changes in concentration and in the total amount of glucose recovered at the end of an hour were so slight that they fall within the range of the experimental error.

In general the peripheral venous blood showed a rise in the blood sugar level whenever an appreciable quantity of glucose was absorbed.

Our data are not in agreement with those of Maddock, Trimble and Carey,[11] who reported that regardless of the concentration of the glucose used absorption from the stomach did not occur.

It may be that interference with the gastric blood supply at the pylorus, which can easily be damaged, may account for the differences obtained by different investigators.

From an analysis of our data we conclude that the absorption of glucose from the stomach bears a relationship to the concentration of the glucose solution used, volume remaining approximately constant.

The rise of the blood sugar level when markedly hypertonic solutions were used is further indication of the fact that the glucose which was lost from the gastric contents was actually absorbed.

Conclusions. Glucose is absorbed from the stomach when present in high concentrations. The rate of absorption would seem to bear a relationship to the concentration of the solution in the stomach.

17 Cori, C. F., *J. Biol. Chem.*, 1925, **66**, 691.

10596

Acceleration of Formol Detoxification of Staphylococcus Toxin by Adsorption.

J. M. Johlin.

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The writer has heretofore presented data[1, 2, 3] which demonstrate the manner in which various biological products are attenuated by interfacial adsorption. The adsorption agents chosen have been such as are relatively inert and can easily be removed after any desired degree of change has been effected. The present paper includes data which demonstrate the acceleration of the formol detoxification of staphylococcus toxin† by adsorption processes under various conditions. Johlin and Rigdon[4] have demonstrated the relative effect produced on staphylococcus toxin by emulsions of chloroform and ether as adsorption agents. In the present paper a comparison is made of the action of chloroform and of emulsified gases, oxygen and nitrogen, on staphylococcus toxin, both in the presence and in the absence of formaldehyde.

The attenuation of staphylococcus toxin by emulsified gases is very much more effective than that of other toxins, for example, ricin, whose detoxification by emulsified gases has been attempted in this laboratory.

The emulsification of the staphylococcus toxin and the adsorption agent was brought about by shaking in sealed pyrex glass tubes by a mechanical shaking device. When chloroform was used, all noticeable traces of this substance were removed by subsequent evaporation under reduced pressure at a temperature below 40°C. To determine the relative hemolytic activity of treated and untreated toxin, decreasing amounts of toxin, 0.5 cc to 0.0001 cc, were diluted to 1 cc with normal saline in a series of test tubes and mixed with 1 cc of a 2% suspension of the thoroughly washed red blood cells of rabbits. The relative degree of hemolysis was observed after standing over night.

* Aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation and by a grant from the American Medical Association.

† Obtained through the courtesy of the Lederle Laboratories.

1 Johlin, J. M., *J. Biol. Chem.*, 1929, **81**, 99.

2 Johlin, J. M., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 523.

3 Johlin, J. M., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 568.

4 Johlin, J. M., and Rigdon, R. H., to be published.

TABLE I.

The relative hemolysis of red blood cells by staphylococcus toxin after treatment of variable periods of time with (a) emulsified chloroform, (b) emulsified chloroform in the presence of .25% formaldehyde, (c) emulsified nitrogen, and (d) emulsified nitrogen in the presence of .25% formaldehyde. 4 indicates complete hemolysis, 0 indicates no hemolysis, and 1, 2, and 3 indicate intermediary stages.

Time of Treatment	Emulsifying Agent	cc of toxin diluted to 1 cc															
		.5	.3	.2	.1	.05	.03	.02	.01	.005	.003	.002	.001	.0005	.0003	.0002	.0001
15 min	Chloroform in an	4	4	4	4	4	4	4	4	3	3	2	1	1	0	0	—
30 "	atmosphere of	4	4	4	4	4	4	4	4	3	3	2	2	1	1	—	—
1 hr	nitrogen without	4	4	4	4	4	4	4	4	3	3	2	1	1	0	—	—
2 "	formaldehyde	4	4	4	4	4	4	4	4	3	2	1	1	—	—	—	—
3 "		4	4	4	4	4	4	4	3	2	2	1	1	—	—	—	—
6 "		4	4	4	4	4	4	4	3	2	2	1	1	—	—	—	—
12 "		4	4	4	4	4	4	3	3	2	2	1	1	—	—	—	—
24 "		4	4	4	4	4	3	3	2	2	1	1	1	—	—	—	—
15 min	Chloroform in an	4	4	4	4	3	3	2	2	1	1	1	0	0	0	0	—
30 "	atmosphere of	4	4	4	4	4	3	3	2	1	1	1	1	0	0	—	—
1 hr	nitrogen in the	2	2	2	1	1	1	1	0	0	0	0	0	0	0	—	—
2 "	presence of .25%	1	1	1	0	0	0	0	0	0	0	0	0	—	—	—	—
3 "	formaldehyde	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
6 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
12 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
24 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
30 min	Nitrogen without	4	4	4	4	4	4	4	4	4	4	4	3	2	2	—	—
1 hr	formaldehyde	4	4	4	4	4	4	4	4	3	3	2	2	1	1	—	—
2 "		4	4	4	4	4	4	4	4	3	3	2	1	—	—	—	—
3 "		4	4	4	4	4	4	4	3	2	1	1	1	—	—	—	—
6 "		4	4	4	4	4	3	3	2	2	1	1	0	—	—	—	—
12 "		4	4	4	4	3	3	2	2	1	1	1	0	—	—	—	—
24 "		4	4	4	3	3	2	2	1	1	0	0	0	—		—	—
30 min	Nitrogen in the	4	4	4	4	4	4	4	4	4	3	3	2	1	0	—	—
1 hr	presence of .25%	4	4	4	4	3	3	3	2	2	1	1	1	0	0	—	—
2 "	formaldehyde	2	2	2	2	1	1	1	0	0	0	0	0	—	—	—	—
3 "		1	1	0	0	0	0	0	0	0	0	0	0	—	—	—	—
6 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
12 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
24 "		0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
None	Control (untreated toxin)	—	—	—	—	4	4	4	4	4	4	4	3	3	2	2	1
12 hr	Standing with	—	—	—	—	4	4	3	3	2	2	1	1	1	1	1	0
24 "	.25% formalde-hyde without emulsification	4	3	3	3	2	2	2	2	1	1	1	1	0	0	0	0

TABLE II

A Comparison of the Detoxifying Action of Emulsified Gases, Oxygen and Nitrogen, with Each Other and with Emulsified Chloroform Both in the Presence and Absence of .25% Formaldehyde.

Time of Treatment	Emulsifying Agent	cc toxin diluted to 1 cc															
		.5	.3	.2	.1	.05	.03	.02	.01	.005	.003	.002	.001	.0005	.0003	.0002	.0001
1 hr	Nitrogen	—	—	—	—	4	4	4	3	3	2	1	1	0	0	0	0
2 "		—	—	—	—	4	4	3	2	2	1	1	0	0	0	0	0
1 "	Oxygen	—	—	—	—	4	4	4	3	2	2	1	1	0	0	0	0
2 "		—	—	—	—	4	4	3	2	1	1	1	0	0	0	0	0
1 "	Nitrogen in the	4	4	4	4	4	4	3	3	2	2	2	1	—	—	—	—
2 "	presence of .25% formaldehyde	2	2	2	2	1	1	0	0	0	0	0	0	—	—	—	—
1 "	Oxygen in the	4	4	4	3	3	2	2	1	0	0	0	0	—	—	—	—
2 "	presence of .25% formaldehyde	2	2	1	1	1	0	0	0	0	0	0	0	—	—	—	—
1 "	Chloroform in an	—	—	—	—	4	4	3	3	2	2	1	1	0	0	0	0
2 "	atmosphere of nitrogen	—	—	—	—	4	4	3	2	2	1	1	0	0	0	0	0
1 "	Chloroform in an	—	—	—	—	4	4	3	2	2	1	1	1	0	0	0	0
2 "	atmosphere of oxygen	—	—	—	—	4	4	3	2	2	1	1	0	0	0	0	0
1 "	Chloroform in an	2	1	1	1	0	0	0	0	0	0	0	0	—	—	—	—
2 "	atmosphere of nitrogen in the presence of .25% formaldehyde	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
1 "	Chloroform in an	1	1	1	1	0	0	0	0	0	0	0	0	—	—	—	—
2 "	atmosphere of oxygen in the presence of .25% formaldehyde	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
None	Control (untreated toxin)	—	—	—	—	4	4	4	4	4	4	4	3	2	2	1	1

TABLE III.

A Comparison of the Relative Detoxification of Staphylococcus Toxin, Containing Varying Amounts of Formaldehyde, by Emulsification with Nitrogen for Different Periods of Time.

Time of Treatment	% Formaldehyde	cc of toxin diluted to 1 cc															
		.5	.3	.2	.1	.05	.03	.02	.01	.005	.003	.002	.001	.0005	.0003	.0002	.0001
2 hr	.25	3	3	2	2	1	1	1	1	1	0	0	0	—	—	—	—
”	.10	4	4	4	4	3	2	2	2	1	1	1	1	—	—	—	—
”	.05	4	4	4	4	4	4	4	4	4	3	2	1	—	—	—	—
”	.01	4	4	4	4	4	4	4	3	2	2	1	1	—	—	—	—
4 hr	.25	1	1	1	0	0	0	0	0	0	0	0	0	—	—	—	—
”	.10	3	3	3	2	2	1	1	1	0	0	0	0	—	—	—	—
”	.05	4	4	4	3	3	2	2	2	1	0	0	0	—	—	—	—
”	.01	4	4	4	4	3	3	2	2	2	1	1	0	—	—	—	—
6 hr	.25	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
”	.10	1	1	1	1	0	0	0	0	0	0	0	0	—	—	—	—
”	.05	4	3	3	2	2	1	1	0	0	0	0	0	—	—	—	—
”	.01	4	4	4	3	3	2	2	1	1	0	0	0	—	—	—	—
Control	Untreated toxin containing no formaldehyde. Not emulsified	—	—	—	—	4	4	4	4	4	4	4	4	3	3	2	1

The relative efficiencies of emulsions of chloroform, oxygen and nitrogen, in detoxifying staphylococcus toxin, and their relative acceleration of the process of formol detoxification of this toxin are illustrated in the accompanying tables. These tables also compare the effect produced by adsorption with that of the slower action of formaldehyde when the toxin is allowed to stand in its presence without emulsification. Staphylococcus toxin is completely detoxified by emulsification with chloroform, in the presence of 0.25% formaldehyde, in about 2 hours and almost equally fast with emulsified gases, as compared with the days required for a similar detoxification when the toxin is allowed to stand with formaldehyde without emulsification.

The fact that emulsified nitrogen acts like chloroform as an adsorption agent in the attenuation of this toxin furnishes proof that the action of emulsified chloroform is that of adsorption and is not due to its solvent action. A comparison of the action of emulsified nitrogen and of emulsified oxygen, both in the presence and in the absence of formaldehyde, indicates that the attenuation of the toxin by oxidation need not be considered as a probability.

A group of rabbits was immunized, in accordance with the procedure of Bulletin B1199 of the National Institute of Health, with staphylococcus toxin which had been shaken with nitrogen in the presence of 0.25% formaldehyde for 8 hours. This material was found to be entirely non-hemolytic and did not produce any observable symptoms of any kind when 0.5 cc was injected intraperitoneally into each of 4 mice. The hemolytic test dose of the original toxin was found to be 0.059 cc when tested with standard staphylococcus antitoxin.

Three rabbits were found to contain less than 0.02 units of antitoxin per cc of serum before immunization. After immunization the number of units of antitoxin per cc of serum was as follows: Rabbit No. 80, 1.6 units; Rabbit No. 81, 3.0 units; Rabbit No. 91, 1.0 unit.

10597

The Effect of the Pulse upon the Flow of Lymph.*

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In the course of some observations upon the receptaculum chyli, we were impressed by the fact that its position is such that it is ideally located for receiving the transmitted pulsations of the aorta. It lies between the aorta and the vertebral column, being intimately attached to the posterior wall of the aorta. The intercostal vessels form its lateral boundaries. A search of the literature shows that others have believed that the pulsations of arteries may be transmitted to the lymphatic system but positive demonstration of this is lacking.

This paper deals with the results of attempts to register pulsations in lymphatic vessels in several locations in dogs.

Large dogs were used in all experiments. In some instances, cream was given 3 hours before beginning the studies. Approximately 0.1 g of morphine was injected an hour prior to the operation. A slow pulse rate was desired and this was accomplished by using morphine. It was supplemented by ether during the operative procedures except in those instances in which the thoracic duct was exposed in the neck, when local anesthesia was employed. Heparin was used as an anti-coagulant in some of the experiments. A glass cannula as large as the vessel would accommodate was placed in the lymphatic trunk and it was connected by a rubber tube to a Jaquet recording tambour with an air valve and the oscillations were recorded. Four different sites were chosen in the various experiments for the exposure of the large lymph vessels. The most satisfactory of these was that in which the upper part of the cysterna chyli and the lower end of the thoracic duct were exposed through a transpleural incision between the 12th and 13th ribs. Positive pressure anesthesia was used in this procedure. A large right angle cannula was inserted into the uppermost part of the cysterna. The cannula was introduced until it was felt that the tip was in the sub-diaphragmatic portion of the cysterna. An air-tight closure of the chest around the cannula was then performed.

Using this method, oscillations in the column of lymph in the tube which were synchronous with the pulsations in the femoral artery could be seen and recorded. These pulsations did not seem to be de-

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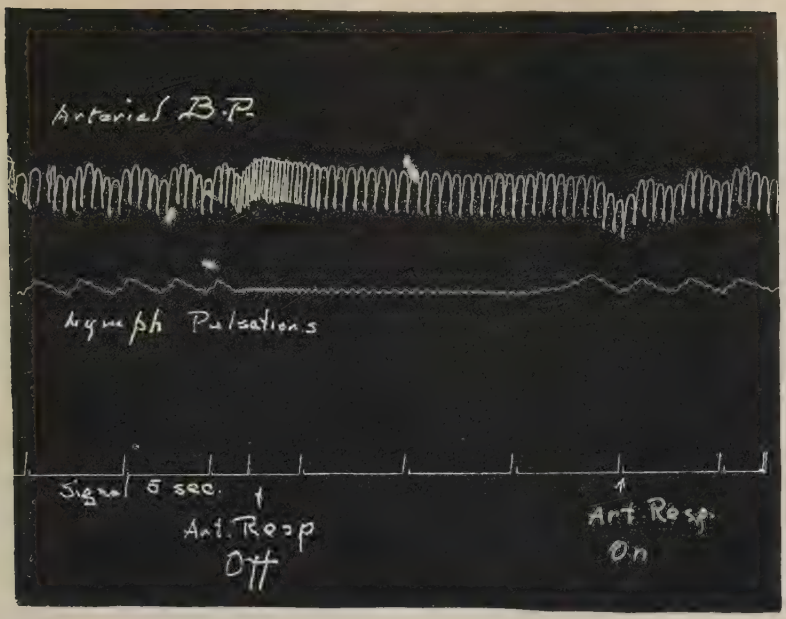

FIG. 1.

Showing the arterial blood pressure and the lymph pulsations as recorded in the receptaculum chyli through a cannula introduced just above the diaphragm. Incision in chest had been closed, animal still anesthetized. Only the oscillations synchronous with the arterial pulse were present when the apparatus producing artificial respirations was disconnected temporarily.

pendent upon the development of back pressure in the lymph trunks. As long as artificial respiration was used and the chest was open, other oscillations were not usually visible. When artificial respiration was suspended temporarily after closure of the chest, no other oscillations appeared. Artificial as well as normal respiratory movements caused definite alterations in the height of the column of lymph when the chest was closed. The effects of the arterial pulsations were superimposed upon these. As stated, the tip of the cannula was located at or below the attachment of the diaphragm. Quiet inspiration was associated with an elevation in the column of lymph and expiration with a decline, exactly the opposite of the alterations in pressure in the pleural cavity. On the other hand, forced expiration resulted in a marked increase in pressure. Some of these findings are shown in Figs. 1 and 2.

Results of a similar character were obtained when the receptaculum chyli was cannulated through an incision in the flank. It was more difficult under these circumstances to prevent leakage around the cannula. An attempt was made to determine whether or not pul-

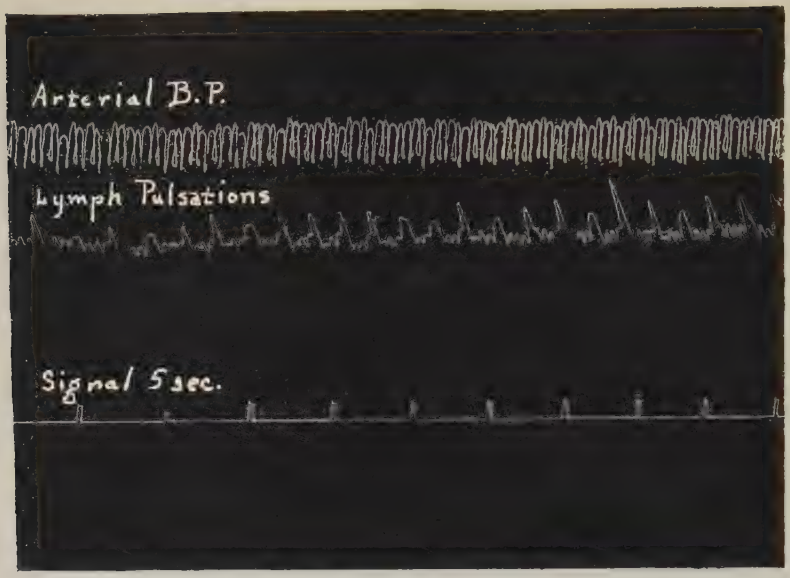

FIG. 2.

Showing the arterial blood pressure and the lymph pulsations as recorded in receptaculum chyli. Animal breathing normally, quiet respirations. The larger alterations in lymph pressure are due to respiratory movements and the smaller ones to arterial pulsations.

sations synchronous with those in arteries are present in the smaller lymph vessels. A cannula which was directed peripherally was placed in a lymph vessel lying anterior to the aorta and similar pulsations were recorded. Due to the proximity of this lymph vessel to the aorta, it is likely that the pulsations were transmitted from it.

Another approach consisted of cannulating the thoracic duct in the neck and similar pulsations synchronous with those in the large arteries were found. This was true also when a vein pocket[1] including the entrance of the duct was used instead of direct cannulation of the duct itself. Ligation of the subclavian artery did not abolish the pulsations. Ligation of the thoracic duct in the lowermost part of the chest caused a diminution but not a total disappearance of the pulsations. Expiration was associated with a rise and inspiration with a fall in the lymph pressure.

According to Poirier, Cuneo and Delamere,[2] the lymphatics are usually grouped together in the neighborhood of blood vessels, which they accompany in almost a straight line. A difference of opinion

1 Lee, F. C., *Am. J. Physiol.*, 1924, **67**, 498.

2 Poirier, P., Cuneo, B., and Delamere, G., *The Lymphatics*, W. T. Keener and Co., Chicago, 1904.

exists as to the agencies responsible for the flow of lymph and as to the relative importance of these. Luciani[3] stated, "At each systolic efflux the whole arterial tree is dilated by the passage of the pulse wave, in consequence of which the whole of the perivascular lymphatics immediately receive an impulse to centripetal evacuation of the lymph which they contain." However, Luciani considered active and passive movements of skeletal muscles and the respiratory mechanism as the most important agencies in promoting the flow of lymph. After enumerating many factors which aid in the flow of lymph, Lee[1] stated that he had observed small fluctuations in lymph pressure, not entirely synchronous with the heart beat, and which he believed to be due to intra-thoracic pressure changes associated with the contraction of the heart. As a result of studies on lymph pressure, Beck[4] stated, "It is probable that the pulsation of the blood-vessels may be transmitted to the adjacent lymph vessels and on account of the numerous valves present in the lymph vessels force the lymph forward and become a factor in the production of the pressures obtained in this study." Sainsbury[5] expressed the belief that the pulse wave is the most important factor in promoting the flow of lymph. Clark and Clark[6] describe the movement of lymph as a "bobbing" back and forth synchronous with the heart beat or respiration, with only a sluggish forward movement.

Since the present experiments were initiated, Parsons and McMaster[7, 8] have reported the results of an extremely interesting study in which it seems to have been proven conclusively that a pulsating stream of blood is an important factor in maintaining a flow of lymph. The ears of rabbits were perfused with defibrinated rabbits' blood in such manner that pulsation could be imported to the perfusate or withheld from it at will. It was found that pulsation of the blood vessels leads to greater formation and flow of lymph, to greater interstitial spread of dye injected into the tissues and to the more rapid absorption of dye. Even though the flow of the perfusate was less and the pressure was lower, pulsating perfusions yielded larger lymph flows and greater spread of dye in the tissues.

The competency of the valves in lymphatics was confirmed by our futile attempts to pass a small catheter through the thoracic duct

3 *Luciani's Human Physiology*, Macmillan and Co., 1911, 1, 519.

4 Beck, C. S., *Johns Hopkins Hosp. Bull.*, 1924, **35**, 206.

5 Sainsbury, Harrington, *The Cardiac Cycle*, William Wood and Co., 1931.

6 Clark, E. R., and Clark, E. L., *Am. J. Anat.*, 1933, **52**, 273.

7 McMaster, P. D., and Parsons, R. J., *J. Exp. Med.*, 1938, **68**, 377.

8 Parsons, R. J., and McMaster, P. D., *J. Exp. Med.*, 1938, **68**, 353.

into the cysterna chyli. It is obvious that the pulsations of arteries would be without influence on the effective lymph flow were it not for the valves which allow the fluid to flow in only one direction. The experiments reported do not allow one to state with certainty that the arterial pulsations are an important agency in promoting the flow of lymph. However, the fact that the lymph pulsations were present even when the ducts did not appear to be distended is highly suggestive. It seems likely that the arterial pulsations are an important factor in promoting the flow of lymph when the subject is completely relaxed or sleeping. Even under these conditions, the respiratory movements aid materially. However, the effect of the respiratory movements is probably exerted in the main on large lymphatic trunks and only indirectly on most of the smaller ones, whereas the arterial pulsations probably influence directly the lymph flow throughout the body. The work of Parsons and McMaster suggests this very strongly. During activity, whether it be of the skeletal or intestinal systems, the muscular movements probably exert a major influence on the flow of lymph. The many factors which promote the flow of lymph vary quantitatively under different conditions.

Summary. Pulsations in lymphatics synchronous with those in arteries have been recorded. The possible effect of the pulse upon the flow of lymph has been discussed.

10598

Optimal Conditions for Recovery of Antibody from Immune Precipitate of Type I Pneumococcus.

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In a previous paper[1] we have shown that the liberation of antibody from immune precipitate of Type I Pneumococcus by the action of dilute alkali is due to a shift of antigen-antibody equilibrium as follows:

$$B_mG_n \underset{\text{neutral}}{\overset{\text{alkaline}}{\rightleftharpoons}} B_{m-x}G_n + xB$$

[1] Liu, S. C., and Wu, H., *Chinese J. Physiol.*, 1938, **13**, 449.

Where G = antigen, B = antibody, and B_mG_n = immune precipitate formed in neutral solution. Upon treatment with alkali, a part of the antibody, xB, is set free from the immune precipitate. A similar shift of equilibrium probably occurs in acid solution. The purpose of the present study is to determine the optimal conditions for the recovery of antibody from the immune precipitate by treatment with acid as well as with alkali.*

Recovery of horse antibody from immune precipitate. Washed precipitate was evenly suspended in water and sixteen 4 cc portions (about 1.2 mg N per cc) were transferred to a series of 15 cc graduated centrifuge tubes. Eight portions were treated with equal volumes of dilute HCl and the other 8 portions with dilute NaOH of different concentrations. The mixtures were allowed to stand 10 minutes at room temperature and centrifuged for 15 minutes. The volumes of the (acid or alkaline) precipitates were recorded and the supernates poured into 16 test tubes. The centrifuge tubes containing the precipitates were allowed to drain on a piece of filter paper.

Four cc each of the supernatants were pipetted into a series of 15-cc graduated centrifuge tubes, each containing 0.6 cc of 10% NaCl and 1 drop of 0.04% phenol red. They were neutralized with dilute HCl or NaOH, and water was added to make a volume of 6.6 cc in each tube. The tubes were centrifuged, the volume of the (neutral) precipitates were recorded, and the supernates were poured into 16 test tubes. The centrifuge tubes containing the precipitate were allowed to drain on a piece of filter paper.

The remaining acid and alkaline supernates were used for pH determinations. On the acid side, glass electrode was used. On the alkaline side, hydrogen electrode was used.

The acid and alkaline precipitates and the neutral precipitates were dissolved with 0.1 N NaOH. Aliquot portions of the resulting solutions and the neutralized supernatants were used for micro-Kjeldahl determinations. Nitrogen of the precipitate was corrected for the N of the supernate left in the precipitate by assuming the volume of fluid in the precipitate to be equal to the volume of the moist precipitate. Results are shown in Table I and Fig. 1.

The acid or alkaline precipitate represents $B_{m-x}G_n$. The neutral precipitate represents dissolved $B_{m-x}G_n$ plus some antibody which it reabsorbed upon neutralization. Nitrogen in the neutralized supernate represents the amount of antibody recovered.

It is well known that the combination of antibody with antigen

* A similar study on the recovery of antibody from the agglutinate is in progress and the results will be reported in another paper.

TABLE I.
Recovery of Type I Pneumococcal Antibody from Horse Immune Precipitate at Different pH's.
t = 23°C.

pH	N in acid or alkaline precipitates, mg	N in neutral precipitates, mg	N in neutralized supernatants, mg	Total N in immune precipitate, mg	Recovery, %
3.48	0.052	4.16	0.784	4.996	15.7
3.73	0.070	4.16	0.694	4.922	14.1
3.86	0.070	4.22	0.720	5.010	14.5
3.97	0.670	3.62	0.754	5.044	15.1
4.25	2.387	0.42	2.292	5.099	44.5
4.50	3.040	0.256	1.610	4.906	32.8
4.66	3.400	0.056	1.560	5.016	31.0
5.20	4.230	0.04	0.610	4.880	12.5
8.91	4.140	0.12	0.880	5.140	17.1
9.56	3.58	0.178	1.286	5.044	26.5
9.77	3.01	0.412	1.730	5.152	33.6
10.01	2.15	1.10	1.760	5.010	35.2
10.09	1.90	1.30	1.904	5.104	37.3
10.60	Negligible	3.72	1.240	4.960	25.1
10.69	"	3.82	1.200	5.020	23.8
10.95	"	4.36	0.784	5.144	15.3

takes place in the pH range from 5 to 9. If pH is below 5 or above 9, combination is not complete.[2] Conversely, if neutral immune precipitate is treated with acid or alkali some antibody should be liberated. However, not all the antibody which is so liberated is recoverable, because on neutralization part of the liberated antibody xB recombines with the dissolved $B_{m-x}G_n$. In solutions not far from neutrality (pH 5-6 and 8-9) where the amount of dissolved $B_{m-x}G_n$ is negligible, practically all the antibody which is set free by acid or alkali is recovered if the $B_{m-x}G_n$ precipitate is removed. In these pH ranges, however, the shift of equilibrium is slight, and the percentage recovery of antibody is low. In more acid or alkaline solutions, the shift of equilibrium is greater, but the amount of dissolved $B_{m-x}G_n$ also increases, which decreases the recovery. At a certain pH where the shift of equilibrium and the solubility of $B_{m-x}G_n$ strike a most favorable balance, the recovery is optimal. Another possible reason for the decrease of recovery in more acid or alkaline solutions is the denaturation of the antibody-protein. However, denaturation is probably not an important factor when the duration of treatment with acid or alkali is as brief as in the experiment here reported.

As shown in Fig. 1, the optimal pH is 4.25 on the acid side and 10 on the alkaline side. It has been noted that the optimal position is the same for immune precipitates prepared with different lots of polysac-

[2] Marrack, J. R., *The Chemistry of Antigens and Antibodies*, His Majesty's Stationery Office, London, 1938, pp. 129, 140.

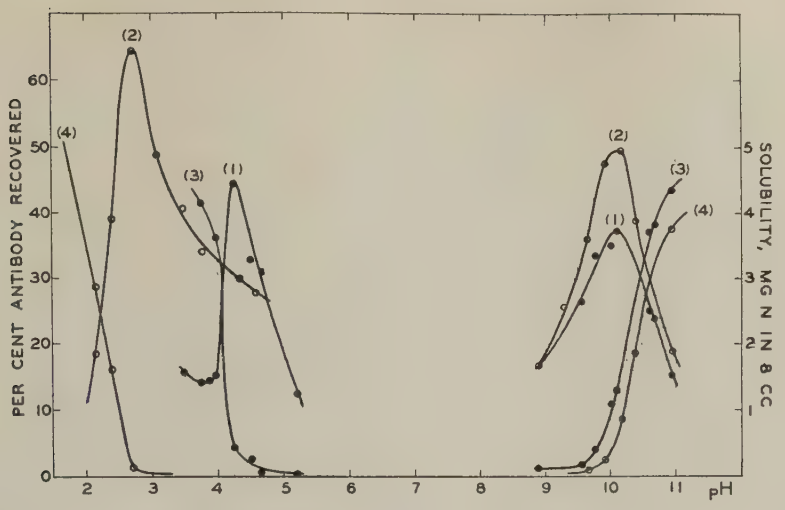

FIG. 1.

Recovery of Type I pneumococcus antibody from immune precipitate at different pH's, t = 23°C.

(1) % recovery without NaCl.
(2) " " with NaCl.
(3) Solubility of acid or alkaline precipitate without NaCl.
(4) " " " " " " with 1% NaCl.

charide, but slightly different for precipitates prepared from antisera of different horses. It has also been noted that the acid or alkaline precipitate is usually loose and translucent at the optimal pH but compact and granular at other pH's. The optimal pH can be roughly located by comparing the relative volumes of the precipitates. The significance of these findings deserves further study.

Effect of NaCl. Washed immune precipitate of Type I pneumococcus was suspended in 2% NaCl and treated with dilute acid or alkali as described above. The results are also plotted in Fig. 1. It will be noted that the solubility of $B_{m-x}G_n$ is considerably decreased by NaCl. For instance, at pH 10, the solubility of the alkaline precipitate decreases from 1.2 to 0.6 mg N in 8 cc, and the recovery increases from 38 to 50%. On the acid side, the effect of NaCl is so marked that the optimal pH is shifted from 4.25 to 2.75 and the recovery increases from 45 to 65%.

By quantitative precipitin-reaction, the purity of the antibody recovered with dilute acid, pH 4.25, and dilute alkali, pH 10, was compared with those recovered by other methods. To 4 cc portions of the antibody- solution (0.25 mg N per cc) were added 1 cc portions of polysaccharide solutions of different concentrations. The precipitates were washed and their N contents determined. Correction

was made for the polysaccharide N in the precipitate. At the point of maximal precipitation the products recovered with dilute acid and with concentrated NaCl[3] were both 91% precipitable, while those recovered with dilute alkali and with alkaline calcium phosphate[4] were 86 and 85% precipitable respectively.

Since proteins are more easily degraded by alkali than by acid, it is to be expected that the antibody recovered with alkali is less pure than that recovered with acid. While the antibody recovered with concentrated salt is as pure as that recovered with dilute acid, the latter method gives a higher yield and requires less time.

Recovery of rabbit antibody from immune precipitate. This precipitate is much more soluble than that of equine origin. In the absence of NaCl, complete solution of the precipitate occurs when the pH is above 8.6 or below 4. For this reason, the recovery is low and shows no optimal point. If the precipitate is suspended in 3% NaCl instead of water, the recovery is much improved. On the acid side there is an optimum at pH 2.70 with a recovery of 64%. On the alkaline side the optimum lies between pH 9 and 10 and the recovery varies between 30 and 50%. This variation is probably due to the fact that rabbit immune precipitate is gelatinous in alkaline solution and does not reach equilibrium as easily as in acid solution.

Summary. There is an optimal pH for the recovery of antibody from immune precipitate of Type I pneumococcus by treatment with dilute acid or alkali. In the presence of NaCl, the percentage of recovery is increased. The mechanism of the recovery is discussed. The present findings furnish the basis of a method for the isolation of antibody which is better than any of the existing methods.

10599

Distribution of Murine Typhus Rickettsiæ in Developing Chick Embryo.

K. H. PANG. (Introduced by Samuel H. Zia.)

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Following the method of Goodpasture, Zia[1] cultivated successfully typhus Rickettsia of endemic and epidemic types in the chorioallantoic membrane of developing chick embryo. This was confirmed by

[3] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1936, **64**, 161.

[4] Felton, L. D., *J. Immunol.*, 1932, **22**, 453.

[1] Zia, S. H., *Am. J. Path.*, 1934, **10**, 211.

Wenckebach[2] with endemic typhus, Bengtson and Dyer[3] with Rocky Mountain spotted fever, and Cox[4] with endemic typhus fever and Rocky Mountain spotted fever. These authors made a special study of the distribution of the Rickettsia in the infected embryo and found that whole embryo, brain, liver, chorioallantoic membrane, and yolk sac were infectious for guinea pigs, thus proving the presence of Rickettsial bodies. On the other hand Bengtson was not able to demonstrate any Rickettsia by direct smear in any of the organs. In view of the fact that various viruses have a different distribution in the fertilized egg, it was considered of interest to study the distribution of Rickettsia of the murine typhus fever by cultural methods. The procedure employed was as follows:

Growth of murine typhus Rickettsia in Maitland culture was inoculated on to the chorioallantoic membrane of fertilized eggs 10-12 days old. They were incubated at 34°C for 8-9 days after which the eggs were opened, and the membrane, liver, spleen, stomach, intestine, brain, lungs, heart, and kidney were aseptically removed and inoculated separately on to Zinsser's "tissue-agar" media[5] and Maitland flasks. Direct smear of all tissues were negative except for a few organisms occasionally found in smears made from the membranes. The cultures were examined after 12-20 days of incubation, and Macchiavello's technic of staining was employed for demonstration of Rickettsia. Sections of brain, liver, and spleen were made and stained with eosin and hematoxylin.

Result: It was found that about 80% of the 48 eggs so inoculated showed dead embryos on examination, of which approximately 10% was found to have died of bacterial contamination. The high mortality recorded may be due to several factors: spontaneous death, accidental injury during inoculation, and toxic effect of the Rickettsial infection. As far as our experience goes about 20% of the control 9-day-old fertilized eggs incubated at 34°C died spontaneously without any apparent cause. Therefore the high mortality of the embryos might be attributed to either trauma or infection. In 2 instances Rickettsia was isolated from the liver of dead embryos and in one instance from all the visceral organs and brain of a dying embryo; the growth was particularly heavy in this case. These findings suggest that some of the embryos actually died of Rickettsial infection. Of the remaining 7 surviving embryos, Rickettsia

2 Wenckebach, G. K., *Z. f. Hyg. u. Infekt.*, 1936, **117**, 358.

3 Bengtson, I. A., and Dyer, R. E., *Pub. Health Rep.*, 1935, **50**, 1489.

4 Cox, Herald R., *Pub. Health Rep.*, 1939, **53**, 2241.

5 Zinsser, H., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 604.

was isolated from various organs, chorioallantoic membrane, liver, spleen, stomach, intestine, brain, lungs, heart, and kidney. The growth of Rickettsia was particularly abundant and regular from liver, while those from the membrane and the brain were poor. In this respect, murine typhus Rickettsia simulates louping ill and influenza virus[6] in producing both a local lesion on the chorioallantoic membrane and a systemic effect. Apart from the increase of fibrous tissue and proliferation of cells in the ectodermal layer of the membrane previously noted, there was evidence of fatty degeneration in the liver and massive eosinophilic infiltration of the spleen, but no pathological change was found in the brain. All these seem to suggest that typhus Rickettsia of the murine type is able to produce a local lesion on the chorioallantoic membrane as well as a generalized infection which often kills the chick embryos.

10600

Cultivation of Gonococcus in Tyrode-Serum Mixture.

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In the preparation of gonococcus polysaccharides, we have encountered much difficulty in growing this organism in large quantities. While several liquid and semisolid media[1–4] have been used to grow this organism in large amounts and to keep its viability for a limited period, none was found to serve our purpose satisfactorily. The best medium is probably that of Singh[5] but its preparation is rather complicated. In the course of our investigation, we have found the following medium to be simple and efficient, not only for mass-production of gonococcus but also for preserving its viability for a long period.

The medium was prepared by simply mixing 4 parts of Tyrode solution and one part of horse or human serum. When properly

6 Burnet, F. M., *Gt. Brit. Med. Res. Council, Rep. Series No.* 220, 7, 1936.

1 Clark, L. T., and Ferry, V. S., *J. Immunol.*, 1931, **21**, 233.

2 Carbus, B. C., *J. A. M. A.*, 1932, **98**, 532.

3 Warden, C. C., *J. Inf. Dis.*, 1913, **12**, 93.

4 Torrey, J. C., and Buckell, G. T., *J. Inf. Dis.*, 1922, **31**, 125.

5 Singh, N., *Ind. J. Med. Res.*, 1934, **21**, 769.

prepared this mixture always gives a pH of 7.8. It was sterilized by filtration through Seitz filter, after which it was put into sterile flasks plugged with sterile rubber stoppers and finally sealed with paraffin before incubation at 37°C for 48 hours to ensure sterility.

The ability of this medium to support the growth of gonococcus was tested with 7 freshly isolated strains and one stock strain of gonococcus. Following inoculation with a suspension of gonococcus derived from these strains and upon incubation at 37°C diffuse granular growth began to appear after 48 hours. On further incubation a considerable portion of the growth appeared to have settled down at the bottom of the flasks. The maximal growth, however, was not reached until the end of a week's incubation. The growths of 7-10 days' incubation at 37°C were found to be rich enough to yield approximately 1 mg of crude gonococcus polysaccharides per 100 cc of the culture. In our experience a similar yield of gonococcal polysaccharides would require the growth from 15 petri dishes (10 cm in diameter) of nutrient agar pH 7.4 containing 5% horse blood. Thus it is well illustrated that the Tyrode-serum medium is suitable for growing the gonococcus in large amounts especially for purpose of preparing gonococcal vaccines, nucleoprotein, and polysaccharides.

The viability of gonococcus at 37°C in this medium contained in sealed flasks was determined by plating on blood-agar medium (pH 7.4, 5% horse blood) at weekly intervals. In 6 of the 7 freshly isolated strains studied, growth on plates was regularly present as long as 2½ months, beyond which no viable organism could be recovered. In the remaining strain the organism was apparently viable even at the end of 3½ months. No change in the morphological character of the organism or colony has been noted during this period of observation. This, therefore, demonstrates clearly that gonococcus can survive in this medium at 37°C for a much longer period than that ordinarily observed with use of other media.

It is to be noted that sealed flasks have been used. The amount of free air contained in these flasks seemed to have an effect on the quantity of the resultant growth. The mechanism involved in this regard is not entirely clear to us, but it is likely that the amount of free CO_2 present may have something to do with the maintenance of an optimal pH for profuse growth.

From these observations it can be said that the Tyrode-serum medium described is a simple and efficient medium for the mass-cultivation of gonococcus and the preservation of its viability at 37°C.

10601

Solustibosan in Treatment of Kala-Azar in Chinese Hamsters.

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In the treatment of kala-azar in Chinese hamsters it was shown[1] that with 3 injections a week more antimony in the form of solustibosan than that in the form of neostibosan was required to bring about a cure. Recently, however, Kikuth and Schmidt,[2, 3] working on the same problem with European hamsters, reported that by increasing from bi-weekly to daily injections, 135 mg of Sb in the form of solustibosan gave the same curative effect as 210 mg of Sb in the form of neostibosan. Hence, they concluded that the former is superior to the latter. Our observations do not, however, seem to agree with theirs, and therefore are here reported.

The present study was carried out at 2 different periods with 2 lots of infected hamsters. Lot A consisted of 28 and Lot B of 20 infected hamsters. The course of treatment in the latter lot was started about 2 months after that in the former lot was concluded. The infective dose of *Leishmania donovani* suspension was the same for all hamsters in each lot and was inoculated intraperitoneally. The presence of infection in the hamsters was proven by liver puncture. Forty-eight infected hamsters were equally divided into 2 groups, each consisting of 14 from Lot A and 10 from Lot B. One group was treated with solustibosan and the other with neostibosan. The duration of infection in the hamsters before the commencement of treatment was 31 days in Lot A and 49 days in Lot B. Neostibosan was freshly prepared each time in a 1% sterile aqueous solution and solustibosan was given undiluted. When an ampoule of the latter drug was not entirely used, it was kept under sterile condition in an ice-chest for use on the ensuing day. A constant daily dose of 6 cc (120 mg Sb) per kilo for solustibosan, and 400 mg (168 mg Sb) per kilo for neostibosan was given subcutaneously. The antimony content in the daily doses of solustibosan and neostibosan was comparable to that used by Kikuth and Schmidt. In Lot A, 2 to 4 hamsters were killed one week* after the completion of a series of 10, 15, 20 and

[1] Wang, C. W., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 418.

[2] Kikuth, W., and Schmidt, H., *Arch. Schiffs. u. Tropen-Hyg.*, 1938, **42**, 189.

[3] Quoted by Schmidt, H., and Peter, F. M., *Advances in the Therapeutics of Antimony*, Georg Thieme, Leipzig, 1938, 206.

* In a previous study,[1] the hamsters were also killed one week after the completion of treatment.

TABLE I.

Relative Value of Daily Injections of Solustibosan and Neostibosan in Experimental Kala-azar in Chinese Hamsters.

Lot of hamsters	Hamster No.	Total No. daily injections	Total dose per Kg in cc	g	mgSb	Leishman-Donovan bodies in spleen: smear	section	Mortality during treatment, %
			Solustibosan.					
A.	2352	10	60		1200	+	++	
	2353	10	60		1200	0	0	
	2354	10	60		1200	++	+++	
	2355	15	90		1800	++	++	
	2356	15	90		1800	++	+++	
	2357	died after 12 injections						
	2358	15	90		1800	++	+++	
	2359	20	120		2400	(+)	(+)	
	2360	20	120		2400	+	+	
	2361	20	120		2400	+	++	
	2362	25	150		3000	++	++	
	2363	25	150		3000	+	++	12.5
	2364	25	150		3000	0	(+)	
	2365	25	150		3000	+	++	
B.	2562	25	150		3000	++	++	
	2565	died after 25 injections						
	2566	25	150		3000	+++	+++	
	2567	25	150		3000	0	0	
	2568	25	150		3000	(+)	+	
	2570	25	150		3000	++	++	
	2571	25	150		3000	+++	+++	
	2572	25	150		3000	(+)	(+)	
	2573	25	150		3000	0	0	
	2574	died after 25 injections						
			Neostibosan.					
A.	2366	died after 4 injections						
	2367	10		4	1680	(+)	(+)	
	2368	died after 9 injections						
	2369	10		4	1680	0	0	
	2370	10		4	1680	(+)	(+)	
	2371	15		6	2520	0	0	
	2372	15		6	2520	(+)	(+)	
	2373	died after 15 injections						
	2374	20		8	3360	0	(+)	
	2375	20		8	3360	(+)	(+)	
	2377	20		8	3360	0	0	
	2378	25		10	4200	0	0	
	2379	25		10	4200	0	0	
	2380	25		10	4200	+	+	
B.	2548	died after 23 injections						25.0
	2550	died after 25 injections						
	2551	25		10	4200	+	(+)	
	2552	25		10	4200	(+)	(+)	
	2553	25		10	4200	0	0	
	2554	25		10	4200	++	++	
	2555	25		10	4200	0	0	
	2556	25		10	4200	+	+	
	2557	25		10	4200	0	0	
	2558	died after 25 injections						

0 = Not found.
(+) = Very few found.
\+ = Found in every 10-20 oil immersion fields.
++ = " " " 5-10 " " "
+++ = " " " 1-5 " " "

25 daily injections; while in Lot B all the hamsters were killed one week after the completion of a series of 25 daily injections. Smears and sections were made from the spleen and examined for parasites. The results of the treatment of Lot B were combined with those of Lot A.

As shown in Table I, in the group of hamsters treated with solustibosan, all except 3 animals still showed a large number of parasites in the sections made from the spleen. On the other hand, in the group treated with neostibosan, 8 hamsters showed no parasites in the sections of the spleen, while the rest showed in most instances only a few parasites. It appears obvious that solustibosan given at the daily dose of 120 mg of Sb for 10 to 25 consecutive days showed a lower rate of cure than neostibosan given at the daily dose of 168 mg of Sb for the same length of time, although the difference was not statistically significant. Further study on a large series of animals for each group might elucidate this point.

Another point of interest which has been brought out from the present study was that for a given total number of injections or a given total dose of antimony in the form of either solustibosan or neostibosan, daily injections appeared to be less effective than bi-weekly or tri-weekly injections. Table II illustrates this point. The data on bi-weekly or tri-weekly injections are those of 2 previous studies[1, 4] and some unpublished observations. It is evident that with increasing frequency of injections there is a decreased rate of apparent cure. The criterion of an apparent cure, as described previously,[4] consists of either a negative spleen smear at autopsy or a negative liver puncture smear as well as a negative spleen puncture smear. With bi-weekly injections, neostibosan has been shown to give an apparent cure-rate nearly twice as high as with daily injections, and the dif-

TABLE II.

Relative Value of Daily, Bi-weekly, and Tri-weekly Injections of Solustibosan and Neostibosan in Experimental Kala-azar in Chinese Hamsters.

Frequency of injections	Solustibosan		Neostibosan		
	Daily	Tri-weekly	Daily	Tri-weekly	Bi-weekly
Dose in mgSb per Kg	120	160-320	168	168	84-378
Total dose in mgSb per Kg	1200-3000	1440-3040	1680-4200	1512-4032	2100-4200
No. of hamsters killed and examined	21	12	18	12	*36
No. of hamsters showing negative spleen smears	4	4	9	10	34
Rate of apparent cure %	19.0	33.3	50.0	83.3	94.4

*Twenty-one of these 36 hamsters were not killed but all showed negative liver and spleen punctures 3 weeks after the completion of treatment.

[4] Wang, C. W., and Lee, C. U., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 674.

ference of apparent cure-rate between bi-weekly and daily injections was found to be statistically significant (44.4 ± 12.5).[5] It appears likely that the decreased effectiveness of neostibosan and solustibosan following daily injections is related to the increased rate of excretion of antimony. Brahmachari[6] has shown that with repeated injections the amount of antimony excreted by the kidneys was fairly proportional to the amount of antimony present in the tissues, and that when the amount of antimony present in the tissues reached a maximum concentration, its excretion suddenly became increased out of proportion to the amount present in the tissues. With daily injections it seems likely that the amount of antimony present in the tissues reaches a state of maximum concentration rather early; otherwise, the cumulative effect of antimony would have manifested itself.

The higher mortality rate in the group of hamsters treated with neostibosan agrees with our previous experience, although the difference may not be significant.

Conclusions. In the treatment of kala-azar in Chinese hamsters it was found that with daily injections of the drugs under investigation, less antimony in the form of solustibosan than that in the form of neostibosan did not produce the same curative effect, as has been claimed by Kikuth and Schmidt, *i. e.*, the curative effect of solustibosan did not increase with the increase of the frequency of injections. 2. Daily injections of either solustibosan or neostibosan appear to be less effective than bi-weekly or tri-weekly injections.

We are indebted to Bayer and Co. for the generous supply of solustibosan used in this and previous studies.

10602

Sensitizing Capacity of Polysaccharide of *Monilia tropicalis.*

T. T'UNG AND SAM C. WONG. (Introduced by Samuel H. Zia.)

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Active sensitization of guinea pigs has been generally successful with gram-positive bacteria but not with gram-negative ones. Yet under suitable methods of preparation polysaccharides derived from some of the latter microörganisms are capable of inducing a state of

[5] Fortuyn, A. B. D., *China Med. J.*, 1929, **43**, 31.

[6] Brahmachari, U. N., and Sen, P. B., *Ind. J. Med. Res.*, 1924, **12**, 113.

supersensitivity in guinea pigs.[1] From this it would appear that polysaccharides prepared from gram-positive organisms should possess sensitizing property comparable to or better than that of the intact cell if a proper method of extraction is employed. On the other hand it is also conceivable that the sensitizing capacity of gram-positive organisms may represent a unique function of the bacterial protein. A gram-positive yeast-like fungus, *Monilia tropicalis*, was thought especially suitable for this study because previous studies[2] have shown that a small dose of killed culture of this microorganism sensitized guinea pigs regularly.

Monilia tropicalis was grown on Sabouraud's medium at 37°C for 48 hours. The organisms were collected and the polysaccharide was prepared by 1% acetic-acid hydrolysis of the whole organism.[3] The polysaccharide in 1% solution gave none of the usual reactions of protein and contained 1.12% total nitrogen. A 1:10,000 dilution of the soluble antigen gave a strong Molisch reaction.

Preliminary studies were made to determine the antigenic activity of the polysaccharide. Two rabbits received intravenously a total of 9 mg of the substance, the method of immunization being the same as that employed elsewhere.[4] Seven days after the last injection the sera of these animals were examined for precipitin and complement-fixing bodies. It was found that both sera reacted equally with a 1:10,000 dilution of the antigen in the former test and with a 1:100,000 in the latter.

Two series of guinea pigs weighing 250-300 g each were employed for the study of active sensitization. The first series consisting of 9 animals received 2 subcutaneous and 1 intraäbdominal injection of polysaccharide at 5-day intervals. The total dose was 21 mg per animal. The second series consisting of 7 animals received 3 intra-abdominal injections of 1 cc each using the same time interval. The total dose was equal to 1:5 the growth of a 24-hour agar-slant. Three weeks after the last injection all animals were tested intravenously with 5 mg of the polysaccharide. The results, presented in the first 2 columns of Table I, show that 2 of 9 animals sensitized with polysaccharide exhibited non-fatal shock. On the other hand, all animals sensitized with the whole organism reacted to the intravenous injection of 2-5 mg of the polysaccharide, 5 anaphylactic deaths having

1 Kurotchkin, T. J., and Wong, Sam C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 113.

2 Lim, C. E., and Kurotchkin, T. J., *Chinese Med. J.*, Suppl. I, 1936, 256.

3 Wong, Sam C., PROC. SOC. EXP. BIOL., AND MED., 1938, **38**, 107.

4 Wong, Sam C., and Kurotchkin, T. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 161.

TABLE I.
Presence of Supersensitivity at Various Time Intervals.

First test	No. animals	Second test	No. animals	Third test	No. animals
Severe shock, recovered after		Mod. shock	1	Death	1
		" "	1	Severe shock	1
1½ hr	1	" "	3	" "	3
Moderate shock, recovered after		No response	4	No response	2*
20 minutes	1				
No response	7				

* 2 died of intercurrent infection after the second test.

been recorded. These results were not included in the table, since no further test was made on the 2 surviving animals.

All the guinea pigs of the first series were retested for the return of sensitivity 2 weeks later. It was found that 5 of 9 animals reacted with moderate but non-fatal shock to the intravenous injection of 10 mg of the polysaccharide. Since the number of sensitive animals has increased, a third testing seems to be indicated. All these animals were tested with the same shocking dose 3 weeks after the second test. It was found that 5 of 7 animals reacted, one typical anaphylactic death having been observed and 4 exhibiting severe but non-fatal shock. The results of the last 2 testings presenting the records of each animal are given in the last 4 columns of the table.

From the above results it is clear that the sensitizing and antigenic properties of the polysaccharide of *Monilia tropicalis* prepared by acetic-acid hydrolysis for guinea pigs and rabbits respectively are decidedly inferior to that of the intact cell. It may seem obvious that the sensitizing capacity of the polysaccharide is low since the weight of intact cells employed compared with that of the polysaccharide is small. Furthermore, large doses of the soluble antigen administered over a comparatively long period of time apparently were necessary to induce a state of supersensitivity in guinea pigs. On the other hand, it is of interest to note that a cellular component of the organism apparently free from proteinous materials *per se* is a complete antigen in the usual immunological sense of the word.

Conclusion. Polysaccharide derived from *Monilia tropicalis* by acetic-acid hydrolysis is capable of inducing active sensitization of guinea pigs and of eliciting the production of antibodies in rabbits. Its capacity in these respects, however, is inferior to that of the intact cell.

10603

Development of *Clonorchis sinensis* Eggs to Cercaria Stage in Laboratory Bred Snails, *Bithynia fuchsiana.*

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Various investigators (Muto,[1, 2] Faust and Khaw,[3] Yamaguti[4]) have in the past described the cercaria of *Clonorchis sinensis* but none of these investigators have given complete experimental proof for their statements. Such proof would seem all the more necessary because these different descriptions did not, on the whole, agree well with each other. Correct knowledge of the cercaria of *C. sinensis* is of special value because with such knowledge it is possible to examine and discover which snail acts as first intermediate host of this parasite.

In order to obtain the cercaria which, beyond question, is that of *C. sinensis,* it is obviously necessary to infect snails with *Clonorchis* eggs and to achieve a full development to the cercaria stage in these snails. Although several investigators (Nagano,[5] Faust and Khaw) realized the importance of this fact, their attempts were unsuccessful so far as complete experimental proof was concerned.

Instead of using old snails collected from rivers and ponds as has been done by former investigators, the present writers used laboratory-bred young snails which may be regarded as not only free from a previous infection with larval stages of trematodes but also as being much more easily infected than older snails. The following is the report of a successful experiment concerning the development of *C. sinensis* eggs to the cercaria stage in laboratory-bred young snails, *Bithynia fuchsiana.*

In the first part of April, 1938, a large number of *B. fuchsiana* were collected in the vicinity of Peiping and kept in an aerated aquarium. Within a few days, eggs of this species of snails were

* The senior author wishes to express his thanks to the China Foundation for the Promotion of Education and Culture for awarding him a research grant to carry out the present work.

1 Muto, M., *Chuo Igakkai Zasshi* (J. Centr. Med. Assn.), 1918, 138, Japanese text.

2 Muto, M., *Ibid.*, 1919, Japanese text.

3 Faust, E. C., and Khaw, O. K., *Am. J. Hyg.*, Monographic Series, 1927, **8**, 1.

4 Yamaguti, S., *Z. f. Parasit.*, 1935, **8**, 183.

5 Nagano, K., *Trans. 6th Congress, F.E.A.T.M., Tokyo*, 1926, **1**, 379.

laid on the glass wall of the aquarium and were immediately removed to another aquarium. Young snails hatched out within a few weeks' time and had already grown to a size of 2-3 mm by the end of May. On June 2nd, a cat, which had been experimentally infected with a large number of *Clonorchis* cysts, was killed and the eggs in the gall bladder were carefully collected and washed. The young snails, together with *Clonorchis* eggs, were put into a small dish which was then covered with fine gauze to prevent the snails from leaving the dish. This dish was carefully put back into the aquarium still covered with the gauze and after one day the gauze was removed and the snails allowed to wander freely in the aquarium. Empty egg shells were found in the feces of the snails which not only showed that the snails had eaten the *Clonorchis* eggs in the dish during the time of their confinement, but probably also that the miracidium in the egg had hatched out in the digestive tract of the snail. The temperature of the water in the aquarium varied from 22°-26°C during the time of the experiment. *Clonorchis* eggs from the gall bladders of other killed cats were put into the aquarium about 15 times during the months of June, July and August in order to afford additional opportunity for the snails to become infected.

On September 11th, 3 months and 8 days after the infection, 2 snails were crushed for examination. One of them was found to be infected. The positive one contained many mature, free swimming cercariae together with many rediae. On September 15th, 3 more snails were crushed. Two contained rediae only and the third one both rediae and cercariae. This showed that the attempt to obtain a complete development of *Clonorchis* eggs to the cercaria stage in the experimentally infected laboratory bred snails, *B. fuchsiana,* had been successful. Consequently the cercariae thus obtained must be regarded as genuine ones of *C. sinensis.*

The cercaria experimentally produced in our snails is an oculate, lophocercous one. The oral sucker is protrusible. On the dorsal margin of the mouth opening there are 4 small, penetrating teeth arranged closely in a horizontal row and, in addition, there are 3 other horizontal rows of minute teeth in that region. The cephalic glands are 14 in number and their ducts are arranged in 4 groups in the formula, $3 + 4 + 4 + 3$, and open into the dorsal margin of the mouth opening. The outer margin of the ventral sucker is not well differentiated. The tail possesses a dorso-ventral fin along its posterior half.

From the above description, it is evident that while the important characteristics of the *Clonorchis* cercaria experimentally produced

in our snails do not agree with those described by Muto and Faust and Khaw, they do agree well with those given by Yamaguti, and therefore we may conclude that Yamaguti's description is certainly based on a study of the genuine cercaria of *C. sinensis.*

Concerning the first intermediate host of *C. sinensis, Parafossarulus striatulus japonicus* was believed to have been incriminated by Muto in Japan, *Melania hongkongensis* by Faust and Barlow,[6] and *Parafossarulus striatulus, P. sinensis, Bithynia fuchsiana* and *B. longicornis* by Faust and Khaw, in China. As proved by the present experiments, *B. fuchsiana* should unquestionably be regarded as one of the first intermediate hosts of *C. sinensis.* Yamaguti's description of the cercaria of *C. sinensis* from *P. striatulus* var. *japonicus* leads us to the conclusion that this also should be regarded as one of its first intermediate hosts. Hsü and Chow[7] have reported *Clonorchis* cercariae in *P. striatulus,* in Canton, identical with those described by Yamaguti, and therefore *P. striatulus* should also be regarded as a first intermediate host of this worm. The result of our experiments indicates that the cercaria described by Faust and his coworkers as being that of *C. sinensis* is, in reality, the cercaria of another fluke; therefore *M. hongkongensis, P. sinensis* and *B. longicornis* cannot be accepted as first intermediate host for *C. sinensis* in China unless definite proof be given that this is true.

10604 P

Type-Specific Polysaccharides of *C. diphtheriae.*

SAM C. WONG AND T. T'UNG. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

Polysaccharides derived from gravis, intermediate, and mitis types of *C. diphtheriæ* were found to be group-specific.[1] Recent findings,[2] however, indicate that any of these different cultural types may be present in a single serological type. Because of this an attempt was made to extend our previous study to include polysaccharides of different serological types of *C. diphtheriæ* with the object of determining the existence of type-specific polysaccharides.

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[6] Faust, E. C., and Barlow, H., *Am. J. Hyg.,* 1924, **4**, 69.

[7] Hsü, H. F., and Chow, C. Y., *Chinese Med. J.,* 1937, **51**, 341.

[1] Wong, Sam C., and T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 422.

[2] Sia, R. H. P., and Huang, C. H., *Ibid.,* in press.

One organism from each of 5 distinct serological types kindly supplied to us by Sia and Huang[2] was employed for the present study. All organisms, with the exception of Park 8 (Type D41) were isolated locally. The various characterizations of these organisms were as follows:

Types	Cultural	Virulence
D14	Gravis	Positive
D25	Mitis	"
D30	Mitis	"
D41	Intermediate	"
X	Mitis	Negative

All of the cultures were capable of eliciting precipitin antibodies in rabbits from 4 to 5 weeks when administered intravenously as heat-killed vaccines. The cultivation of organisms, the preparation of polysaccharides, and the immunization of rabbits were the same as those described previously.[1] Absorption test was performed by mixing immune serum diluted 1:5 with well-washed living organisms grown on serum broth at 37°C for 48 hours and the mixture was incubated at 45°C waterbath for 2 hours. It was refrigerated overnight after which the serum was separated by rapid centrifugation.

It was found that 2 kinds of polysaccharide could be demonstrated by the precipitin reaction and by absorption tests. These polysaccharides will be designated as A and B. Polysaccharide A was present in the types D25, D30, D41, and X. Identical precipitin titers varying from 1:50,000 to 1:100,000 were obtained when polysaccharides A were mixed in the ring test with the homologous as well as the heterologous immune sera prepared with the above types. Cross absorption tests performed by mixing immune serum with organisms belonging to any of the types D25, D30, D41, and X showed a removal of all precipitins without affecting the type-specific agglutinins. Similar treatment of the same immune sera with the organism belonging to polysaccharide B (Type D14) removed neither precipitins nor agglutinins. These findings indicate that at least one of the common antigenic factor among the types D25, D30, D41, and X is the polysaccharide. It might also be mentioned that the polysaccharides obtained from various cultural types reported previously[1] all reacted with the above sera.

On the other hand, polysaccharide B derived from Type D14 diluted 1:100,000 reacted with its homologous antiserum only. No reaction was observed when the same polysaccharide was tested against antisera types D25, D30, D41, and X. Similar negative result was obtained when the antiserum type D14 was tested with

polysaccharides A or with the polysaccharides reported previously.[1] Likewise, cross absorption tests performed by mixing the same serum with organisms belonging to any of the heterologous types did not effect the precipitin titer nor the type-specific agglutinins.

The above findings indicate that 2 distinct kinds of polysaccharide are present in the so-called different serological types included in this study. One type of polysaccharide (A) appears to be shared by at least 4 of the 5 types while the second (B) has been encountered only once. The question of whether or not more than 2 types of polysaccharide are present in other serological types of *C. diphtheriæ* is being investigated.

10605

Bacteriophage Typing of *B. typhosus* Isolated in Peiping.

C. H. YEN. (Introduced by C. E. Lim.)

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Four serologically distinct types of Vi-phages (Type I, II, III, and IV) specific for V-form strains of *B. typhosus* have been described.[1, 2] Type II Vi-phage was found to exhibit a highly selective lytic activity for the strain of *B. typhosus* from which it was propagated. Utilizing this selective behavior of the Type II Vi-phage a method has been evolved whereby V-form strains of *B. typhosus* could be divided into the typical V-forms and the Imperfect V-forms. The typical V-forms were further subdivided into several distinct types (A, B1, B2, C, D1, D2, E, F1, F2, G, H, J).[2] Studies on 706 strains of *B. typhosus* isolated in Canada, England, Norway, Sweden and Denmark have demonstrated the validity of such a scheme of typing.[3] In order to test further the applicability of this scheme of bacteriophage typing and to determine the incidence of different types of *B. typhosus* occurring in this locality, a study was made on 79 strains isolated in Peiping. The results together with an account of isolation of 2 new type strains are given below.

Seventy-nine strains of *B. typhosus* isolated from active cases and carriers during 1937-1939 were inoculated in semisolid agar media

1 Craigie, J., and Yen, C. H., *Trans. Roy. Soc. Canada*, 1937, **31**, Sect. V, 79.
2 Craigie, J., and Yen, C. H., *Canad. Pub. Health J.*, 1938, **29**, 448.
3 Craigie, J., and Yen, C. H., *Canad. Pub. Health J.*, 1938, **29**, 484.

and kept in the refrigerator (0°-6°C). Subculturing was carried out every 3 months. In the present study these stock cultures were plated out on nutrient agar plates (1.5% agar, pH 7.6) and single colonies were picked and employed for typing. These cultures thus derived were then stocked in the nutrient agar stubs, instead of semisolid agar. The original method[2] of typing with Type II Vi-phage preparations* was strictly followed.

Out of a total of 79 strains studied 74 strains of V forms were isolated. Of these 74 strains 23 belonged to the "Imperfect V-forms" and 51 to the typical V-forms. The distribution of the types in these 51 strains are given in Table I. It is to be noted that among the typical V-forms isolated in Peiping only A, D1, and E types were encountered. In addition 2 new types (P16 and P15) were isolated.

Both P15 and P16 strains were found to be insusceptible to the critical phage dilution test[2] of known standard Type II Vi-phage preparations. The Type II Vi-phage was propagated on P16 and P15 separately for numerous generations until the initial phage had been actually diluted beyond 10^{31} folds. These phage preparations were designated as α16 and α15 respectively in accordance with the nomenclature used before.[2]

The critical dilution[2] of 16 was unable to cause a complete area of lysis on plates in any of the known type cultures except Types A and P16. Therefore P16 represents a new type. Subsequently it was found that 6 other local strains belonged to the P16 type.

TABLE I.
Distribution of the Types of *B. typhosus*.

	Typical V types					Imperfect V-forms	W-forms*	Total
	A	D1	E	P15	P16			
No. of strains (a)	11	5	24	4	7	23	5	79
No. of individuals (b)	8	4	12	3	6	11	5	49
Times isolated per individual (c)								
1	5	3	6	2	5	7	5	33
2	3	1	3	1	1	2		11
3			2			1		3
6			1					1
9						1		1

(a) No. of strains belonging to each type.
(b) No. of individuals from whom the above strains were derived.
(c) No. of individuals from whom the isolation has been made.
*Strains of *B. typhosus* devoid of Vi antigen and insusceptible to Vi-phages.

*We are indebted to Dr. James Craigie of the Connaught Laboratories, Toronto, Canada, for supplying us the original stock of the standard "V" Type cultures and the Type II V-phage preparations employed in this study.

TABLE II.
Lytic Activity of α15.

Culture types	Phage Dilutions			
	10^{-2}	3×10^{-2}*	10^{-3}	10^{-4}
P15	C	C	++++	+++
A	C	C	C	C
B1	C	C	C	+++
B2	C	+++	++	+
C	C	C	C	+++
P16	C	C	++	+

C = Whole area lysed.
++++ = Isolated plaque numerous.
+++ = 25-50 plaques.
++ = 20-25 plaques.
+ = less than 10 plaques.
* = critical dilution of α15.

When the critical dilution of α15 was tested on the known standard type strains, it was able to cause a complete area of lysis on the plates not only for Types A and P15 culture but also for B1, C, P16 and to a lesser degree B2, E, G, and H cultures. In order to determine the relationship of α15 to other standard Type II Vi-phage preparations, serial dilutions of this phage were tested on the strains of standard type that are comparatively susceptible to this phage. The results are given in Table II.

It is obvious that α15 seem to be more potent for the cultures of Types A, B1, and C than for P15. This is due to the fact lysis of P15 by α15 results in tiny plaque formation and the lysis is delayed. In fact the lysis becomes more pronounced when the plate cultures of P15 instilled with α15 was first incubated at 37°C overnight followed by leaving at room temperature for another 24 hours. On account of delayed lysis and small size plaque formation the critical dilution of α15 is only as dilute 3×10^{-2}. Thus the properties of P15 and α15 do not resemble any of the known types reported.[2] It was, therefore thought convenient to designate P15 as a distinct type for differentiation from others. Subsequent typing of the local strains revealed that 3 other strains belonged to this new type.

Strains of *B. typhosus* isolated from the same individuals at different times always fall into the same type. Thus a patient (H. 35952) consistently gave isolation of E type strains 6 times and another patient (H. 64490) gave isolation of "Imperfect V form" strains 9 times. Similarly V forms of *B. typhosus* obtained from various sources (stool, blood, urine, bile, rectal swab and chest-wound swab) from the same individual always belong to the same type.

These results clearly indicate the practicability of the method for typing *B. typhosus* by bacteriophage.[2] It is of interest to note that relatively fewer types of typical V form strains were encountered in this locality in comparison with those reported in other countries. Studies are being continued to define the two new type strains (P16 and P15) described.

10606

Type Stability to Bacteriophage of Variants of *B. typhosus.*

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During a study made on the typing of local strains of *B. typhosus,* colonies of various appearances were noted on plating of these old cultures. This afforded the opportunity of determining by the bacteriophage-typing technic[1] whether or not various kinds of colonies derived from a parent stock culture would belong to the same type of V-forms. The observations made regarding this point are herewith communicated.

A collection of 79 strains of *B. typhosus* reported previously[2] was studied. These cultures were kept in semisolid agar media for 3 months to 1½ years with subculturing every 3 months. Upon spreading of these stock cultures on nutrient agar plate (1.5% agar, pH 7.6) followed by incubation at 37°C for 16-20 hours the following kinds of colonies were encountered:

A. Normal Colonies.
   1. Round Margin, Opaque.
B. Variant Colonies.
   2. Round Margin, Translucent.
   3. " " Mosaic.
   4. Rough " Opaque.
   5. " " Translucent.
   6. " " Mosaic.

All normal and variant colonies have smooth surfaces and their suspensions in 0.9% saline are stable. They, however, vary greatly in regard to the colony outline, opacity and size. Thus the colony outline may be smooth and round or irregular, rough with fan-like

1 Craigie, J., and Yen, C. H., *Canad. Pub. Health J.*, 1938, **29**, 448.

2 Yen, C. H., PROC. SOC. EXP. BIOL. AND MED., 1939, in press.

margin, the opacity may be opaque, mosaic or with varying degrees of translucency and the colony size may vary from 1 to 5 mm in diameter. On repeated subculturing the variant colonies showing irregular margins may become smooth and round, and the translucency or mosaic appearance may revert to that of normal opacity. Thus no complete or stable rough colonies have been encountered. These colonies may be regarded as colony variants probably degradation of S form with varying degrees of roughness.

Of 79 strains studied, 27 strains gave only the normal colonies, 3 strains only the variant colonies and the remaining 49 strains gave mixtures of normal colonies with one or more kinds of variant colonies. The result of typing of the normal colony strains has been analyzed in a previous communication.[2] The 3 strains that gave only the variant colonies were also included in that report. These 3 strains were typed on the basis of the sensitivity to the Type II Vi-phage preparations and were found to belong to an Imperfect V form, a Type A type and a P15 type separately.

The results of typing of the normal and variant colonies occurring together from 49 strains of stock cultures are summarized in Table I.

From Table I it is to be noted that when the normal colonies fall into the W, VW, or Imperfect V forms, the variant colonies derived from the same culture also fall into the same forms. But when the normal colonies are of typical V forms, the variant colonies may belong to W, VW, or typical V forms. All the V forms derived from the variant colonies, however, when tested with Type II Vi-phage preparations, were found to belong to the same type as that of the normal colonies, arising from the same culture. There was not a single instance in which the normal colony is of one typical V type and

TABLE I.
Distribution of Types Found in 49 Strains Giving Both Normal and Variant Colonies.

No. of strains	Normal colony	Variant colony
3	W form	W form
17	Imperfect V form	Imperfect V form
2	VW form (Type A and W)	VW form (Type A and W)
3	Type A	W form
5	" A	Type A
1	" D1	VW form (Type D1 and W)
1	" D1	Type D1
3	" E	W form
8	" E	Type E
1	" P15	VW form (Type 15 and W)
2	" P15	Type P15
1	" 16	" W form
2	" P16	" P16

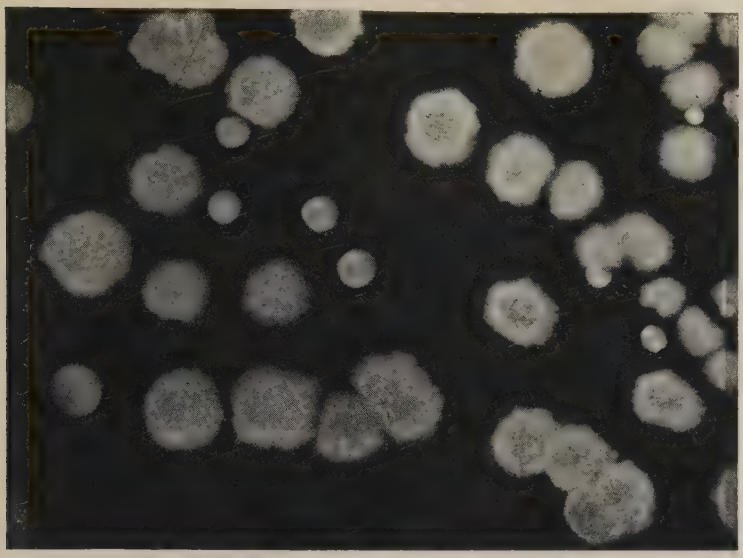

FIG. 1.

Strain P4. 24 hours growth of *B. typhosus* on nutrient agar at 37°C. Variant colonies larger than normal one. Note irregular margin and mosaic appearance of the variant colonies. × 4.

the variant colony of another typical V type. Nor was there any instance in which the variant colony falling to one typical V type while the normal colony from the same culture falling to W or VW forms. Illustrations are given in Fig. 1 showing a mixture of normal and variant colonies belonging to Type A derived from the stock culture P4.

After reversion of variant colonies to normal appearances on subculturing, the type identity of the cultures remained unchanged. It may be pointed out here that the colony appearance is not a reliable guide to indicate whether a colony is a V form or a W form. But W forms are more frequently encountered in the variant colonies than in the normal colonies.

Thus it is clear from these observations that the change of colony appearance from normal smoothness to various degrees of roughness may or may not be accompanied by a loss of the Vi-phage susceptibility. As long as the Vi-phage susceptibility is present in the variant colonies they can be typed with the bacteriophage technic proposed by Craigie and Yen.[1] The fixity of the types defined by the method is well borne out by the fact that the variant colonies when still susceptible to the Vi-phage always fall into the same type as the normal colonies derived from the same culture.

10607

Stability of Mosquito Venom *in vitro*.

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In 1929 the senior author[1] extracted a toxic substance from macerated salivary glands of the yellow fever mosquito, *Aedes aegypti* Linnaeus. At the close of the experiments which were conducted then, a few milliliters of the venom extract remained. This aliquot was passed through a bacterial filter, and a very small amount sealed in glass, and stored in a refrigerator.

On October 14, 1937, the tube was removed from storage. Inspection showed that the filtrate had remained water-clear with no evidence of a precipitate. The glass seal was broken and 0.1 cc of the contents was injected, intradermally, over the biceps muscle of each of 3 persons. Each injection of the salivary gland extract immediately was followed by a control injection of 0.1 cc of sterile saline over the same muscle on the same arm. The pertinent histories and reactions of the subjects are as follows:

Subject No. 1. E.B.M. always had been sensitive to the bite of *Aedes aegypti*. He participated in the experiments of 1929 and showed a strong reaction to the freshly prepared venom extract. Eight-year-old salivary gland extract produced an urticating wheal that persisted. It very closely paralleled the reaction described in 1929 for the same person.[1]

Subject No. 2. Mr. P., who had worked during the previous summer on a mosquito survey project in the Tennessee Valley, supposedly had acquired some immunity. He showed a mild reaction to the test injection, which disappeared completely within 45 minutes.

Subject No. 3. F.C.B. frequently had allowed colonies of *Aedes aegypti* and very closely related species to feed upon his person. Most of this mosquito feeding took place during the years from 1933 to 1936. The feeding of *A. aegypti* upon his arm produced no reaction. The bleb caused by the injection of this 8-year-old venom extract disappeared just as rapidly as did that of the saline control. Although the subject was carefully observed for a possible delayed reaction, none was detected.

Therefore, it would seem that, after 8 years of storage, this salivary extract from *Aedes aegypti* still retained toxicity for those who react to the fresh toxic substance of the yellow fever mosquito.

[1] McKinley, E. B., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 806.

10608 P

Experimental Intersexuality: Modification of Sexual Development of the White Rat with a Synthetic Estrogen.*

R. R. Greene, M. W. Burrill and A. C. Ivy.

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The authors have previously reported on the modifications of embryonic sexual development obtained by injecting large doses of estrogens into pregnant rats.[1-3] The naturally occurring alpha estradiol and its diesterified derivative, estradiol dipropionate, were used in this work.

Diethyl stilboestrol (4,4′ dihydroxy-alpha-beta-diethylstilbene), which is chemically somewhat dissimilar to the naturally occurring estrogens, has been shown to possess many of their functions such as vaginal cornification, growth of the uterus and mammary glands,[4] inhibition of lactation,[5] restoration of pituitary castration changes,[6] prevention of implantation of fertilized ova[7] and inhibition of the effects of progesterone on the uterus.[7]

It seemed of interest to determine if this synthetic substance would have the ability of the natural estrogens to modify embryonic sexual development. Accordingly 40 pregnant rats were given large doses of diethyl stilboestrol† subcutaneously in oil solution. Administration was started in all cases on the 12th or 13th days of pregnancy and continued until the 20th or 21st day, or until resorption of the pregnancies was evident. Twelve of these treated animals carried their pregnancies to term (22nd day of pregnancy). The total dosage these animals received varied from 12 to 42 mg. Eighteen male and 28 female offspring were obtained.

The findings in these newborn are essentially identical with those obtained with the natural estrogens. The external genitalia of both males and females are of the female type and nipples are present in

* Supported in part by a grant from the Josiah Macy, Jr., Foundation.

1 Greene, R. R., Burrill, M. W., and Ivy, A. C., *Science*, 1938, **88**, 310.

2 Greene, R. R., and Burrill, M. W., *Am. J. Physiol.*, Proceedings, 1939, p. 94.

3 Greene, R. R., Burrill, M. W., and Ivy, A. C., *Anat. Rec.*, 1939, **73**, in press.

4 Dodds, E. C., Lawson, W., and Noble, R. L., *Lancet*, 1938, **234**, 1398.

5 Folley, S. F., and Watson, H. M. S., *Lancet*, 1938, **235**, 423.

6 Jacobsen, E., *Endokrinologie*, 1938, **21**, 20.

7 Parkes, A. S., Dodds, E. C., and Noble, R. L., *Brit. Med. J.*, 1938, **1**, 535.

† Furnished by Dr. J. A. Morrell of E. R. Squibb and Sons and by Dr. C. O. Miller of Lakeside Laboratories.

all. Normally nipples are never found in the males of our colony and do not appear in the female until the 2nd to 4th day post partum.

In the female offspring the uteri are large and distended, the ovarian capsule does not develop and the gonads are bare.

Examination of 6 animals by serial sections has revealed changes similar to those obtained with the natural estrogens. In the males, prostates are absent, seminal vesicles, epididymides and vasa deferentia are inhibited and portions of the uteri are present. The upper (Müllerian) vagina is well developed; the lower vagina (that part which is derived from the urogenital sinus) is partially developed. In the females there is some persistence of the Wolffian ducts (cranial and caudal remnants) and some inhibition of the lower vagina.

From these results it is apparent that compounds other than the natural estrogens are capable of modifying embryonic sexual development in the rat.

10609

Growth-Stimulating Action of Ferric Chloride-Treated Wheat Germ Oil.*

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Several investigators have reported the existence of a growth-promoting factor, necessary for rats, that was either vitamin E or a substance closely related to it.[1-4] The pure substance, α-tocopherol, stimulates growth in rats which have plateaued in weight on a vitamin E-low diet.[5] The possibility that more than one factor might possess this growth-stimulating action was shown by Martin,[6] who

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1 Evans, H. M., *J. Nutr.*, 1928, **1**, 23.

2 Blumberg, H., *J. Biol. Chem.*, 1935, **108**, 227.

3 Olcott, H. S., and Mattill, H. A., *J. Biol. Chem.*, 1936, **114**, lxxvii.

4 Olcott, H. S., and Mattill, H. A., *J. Nutr.*, 1937, **14**, 305.

5 Emerson, G. A., and Evans, H. M., *J. Nutr.*, 1937, **14**, 169.

6 Martin, G., *J. Nutr.*, 1937, **13**, 679.

was apparently able to separate the growth-promoting factor from the fertility-restoring factor. He suggested, however, that the apparent difference might be quantitative.

Young rats, suckled by mothers with minimal stores of vitamin E, usually develop a characteristic muscular dystrophy at the end of the lactation period. Goettsch and Ritzmann[7] have found that protection against the dystrophy was afforded by wheat germ oil extracted from a wheat germ from which the antisterility vitamin had been inactivated by $FeCl_3$, and by α-tocopherol. They concluded that the activity of a preparation in preventing muscular dystrophy did not necessarily correspond to its antisterility potency.

Experimental. The results reported deal with the growth-stimulating action of $FeCl_3$ treated wheat germ oil as compared with the original oil. The oils were fed as supplements to female rats of the Long-Evans stock that had plateaued in weight on a vitamin E-free regimen.

The wheat germ oil was treated with a 1% ether solution of $FeCl_3$, according to the method of Waddell and Steenbock.[8] One hundred cc of the oil was shaken for 1 hour in an open flask with 100 cc of a 1% ether solution of $FeCl_3$ at about 50°C; the 2 solutions were thenceforth left together. Residual traces of solvent were removed *in vacuo*. The treated oil was less viscous than the original oil and had a sharp odor. It was kept at room temperature.

Forty-two female rats were placed at 21 days of age on the standard E-low diet 427.[5] They plateaued at an average weight of 232 g after approximately 120 days on the diet. At 145 days of age they were divided into 3 groups of like average weights, as follows:

Group 1—no supplement (controls).

Group 2—80 mg wheat germ oil 6 times weekly.

Group 3—80 mg $FeCl_3$ treated wheat germ oil 6 times weekly.

After receiving the supplements for 50 days the average gains in the wheat germ oil and the $FeCl_3$ treated wheat germ oil groups were approximately the same (Table I).

TABLE I.

Growth Responses of Rats Maintained on a Vitamin E-Low Diet to Supplements of Wheat Germ Oil and $FeCl_3$ Treated Wheat Germ Oil.

Treatment 6 times weekly	No. of rats	Days Supplemented	Avg gain in wt, g
None (controls)	12	145-195	2
80 mg wheat germ oil	15	"	30
80 mg $FeCl_3$ treated wheat germ oil	15	"	28

7 Goettsch, M., and Ritzmann, J., *J. Nutr.*, in press.

8 Waddell, J., and Steenbock, H., *J. Biol. Chem.*, 1928, **80**, 431.

TABLE II.
Vitamin E Activity of Wheat Germ Oil and $FeCl_3$ Treated Wheat Germ Oil.

Material	Level fed, g	No. of rats fed	No. of litters	% littering	Avg No. living young per litter	Avg wt, g	Dead young
Wheat germ oil	0.5	6	6	100	8.0	5.6	1
Same oil treated with $FeCl_3$	2.0	4	0	0	0	0	0
Same oil treated with $FeCl_3$	4.5	7	2	29	3.0	4.2	2

These findings have 2 possible interpretations: that more than one substance possesses growth-stimulating activity or that an amount of vitamin E sufficient for growth in the quantity fed, remained in the oil. If the latter were the case, the amount of the untreated oil fed was then in excess of the requirement.

The $FeCl_3$ treated wheat germ oil had been assayed for vitamin E activity in a single dose of 2.0 g and was found completely inactive although the original oil enabled 6 out of 6 rats to bear living young when fed at a single dose of 0.5 g. It appeared advisable to assay the $FeCl_3$ treated wheat germ oil at an even higher level, namely, at 4.5 g. One-half the oil was given on the first day of the test gestation and the remainder on the second day. Undersized litters of underweight young resulted in 2 out of 7 pregnancies (Table II).

Thus it would appear that the treated oil retained a small percentage of its original vitamin E activity. The results obtained do not permit a quantitative comparison, but it would appear that about one-tenth of the original vitamin E activity of the oil remained in the treated preparation. The rats tested for the growth-promoting properties of the $FeCl_3$ treated oil were therefore receiving only the equivalent of approximately 8 mg of the original oil in terms of its vitamin E activity. If the assumption is made that 500 mg of wheat germ oil is equivalent to 3 mg of α-tocopherol, then the quantity of vitamin E fed in 80 mg of the treated oil would be the equivalent of 0.05 mg of α-tocopherol.

The possibility that more than one substance possesses growth-stimulating activity is not ruled out. It would appear that the feeding of low levels of α-tocopherol will either enable us to establish a low quantitative need for this substance for normal late growth or let us refer the favorable effects of $FeCl_3$ treated wheat germ oil to some constituent other than vitamin E.

Summary. $FeCl_3$ treated wheat germ oil containing approximately one-tenth of the E activity of the original oil stimulated growth when fed at a level of 80 mg six times weekly to female rats which had plateaued in weight on a vitamin E-low diet.

10610

Complement-Fixation Test with Tissue-Culture-Antigens as Aid in Recognizing Latent Avian Psittacosis (Ornithosis).

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The importation of tropical psittacine birds from South America and Australia is always connected with considerable risk in bringing psittacosis to zoölogical gardens, pet stores, aviaries, and breeding establishments of fanciers[1, 2, 3] since latent infections, which may relapse or become activated in transit, are fairly common. As a rule, the existence of psittacosis in a shipment is established by autopsies and the inoculation of mice with the spleens of the few birds which succumb while held in quarantine. Present regulations permit the prompt release of all birds of a consignment provided no deaths are noted in the flock for 2 weeks. On the other hand, it has been fully appreciated that apparently healthy birds may be active or potential shedders of virus. Under the circumstances, it has recently become customary to destroy the entire shipment. Valuable and expensive birds, which on autopsy proved to be free from psittacosis, were thus sacrificed unnecessarily since no methods had been available to segregate the infected from the non-infected. Experiments recently conducted on a shipment of Australian parrots indicate that the complement-fixation test may detect carrier birds with a high degree of certainty.

The complement-fixation tests for psittacosis, originally introduced by Bedson[4] and proven invaluable in the early diagnosis of human infections has been adopted with certain modifications for the examination of parrots. As antigens, concentrated and trypsin-digested cultures of the psittacosis virus in Rivers-Li media have been employed. The preparation of these antigens is briefly as follows: To a medium consisting of 42.5 cc of Tyrode solution and 2.5 cc of chick embryonic tissue fragments (5 cc Tyrode solution to each 11th-day decapitated chick embryo) are added 5 cc of passage-culture, held in 250 cc Erlenmeyer flasks, cotton-plugged and sealed during incubation with "parafilm". After 3 days' incubation, the culture reveals

1 Levinthal, W., *Lancet*, 1935, May 25, 1207.

2 Morgan, M. T., *Bull. Off. Inter. d'Hygiene Publique*, 1938, **30**, 1252.

3 Troup, A. G., Adam, R., and Bedson, S. P., *Brit. Med. J.*, 1939, **1**, 51.

4 Bedson, S. P., *Lancet*, 1935, **2**, 1277; 1937, **2**, 1477.

TABLE I.
Complement-Fixations with Sera of Infected and Healthy Australian Parrots.

No.	History of parrot	Virus demonstrated	Complement fixation reaction		
			Tissue culture Australian virus	Tissue culture California virus	Tissue culture control
1	*Kakatoe galerita*	0 I	1:16++++; 1:16++++	1:8++++; 1:4++	0
2	*Kakatoe galerita*	Spleen	>1:32; 1:32++++	>1:32++++; 1:32	0
4	*Kakatoe galerita*	Spleen	1:64++++; 1:64+++ 1:128+++; 1:128+	1:64++++ 1:128+	0
5	*Kakatoe galerita*	0 I	>1:64++++	>1:64++++	0
6	*Kakatoe galerita*	Spleen	>1:128; >1:128++++	>1:128++++	0
7	*Kakatoe sanguinea*	Spleen	1:16++++; 1:16+++ 1:64++; 1:64±	1:32++++; 1:32+++ 1:64+++	0
11	*Kakatoe roseicapilla*	0	0	0	0
14	*Kakatoe roseicapilla*	0 I	1:16±; 1:10++	1:16++; 1:10++	0
15	*Kakatoe roseicapilla*	0	0	0	0
17	*Kakatoe roseicapilla*	0	0	0	0
20	*Nymphicus hollandicus*	0	1:8+++; 1:10++	1:32++++; 1:10++	0
24	*Nymphicus hollandicus*	Spleen and liver	1:64++++; 1:16++++	1:64++++; 1:32++++	0
29	*Kakatoe sanguinea*	0	0	0	0
30	*Kakatoe roseicapilla*	0	1:8+++; 1:2++	1:8++++	0
31	*Platycercus elegans*	Spleen	1:16++++; 1:16±	>1:32++++; 1:32++++	0

an abundance of elementary (L.C.L.) bodies; only cultures rich in virus should be used. It is shaken mechanically for 15 minutes; centrifuged in horizontal position for 15 minutes at 3,000 r.p.m.; the supernatant fluid is then spun in an angle centrifuge for 1½ hours at 4,000 r.p.m. The sediment is suspended in buffered physiologic salt solution (McIlvaine M/50) and steamed for 30 minutes. An antigen with greater binding power may be obtained by digesting the sediment from the angle centrifugation after which thorough washing will yield a suspension rich in virus particles. They are, as a rule, suspended in 2/5 of the original volume of buffered salt solution (M/50, pH 7.6) and steamed for 30 minutes.

The sera are obtained by bleeding the ether-anesthetized parrots from the ulnar vein *(V. cutanea ulnaria)* of the wings. To prevent premature clotting, it is advisable to cover the disinfected skin area over the vein with a few drops of 1% heparin solution and to use dry (20 gauge 1½ inches long) needles and syringes. The sera inactivated at 56° for 30 minutes are diluted with saline as required; then 0.25 cc of each is placed in sterile tubes, 0.25 cc of antigen added, then mixed with 0.1 cc of complement (titrated and diluted to contain 2 hemolytic units in 0.1 cc) and 0.4 cc of saline. The tubes are shaken and incubated in a waterbath at 37°C for 2 hours; 0.5 cc of sensitized cells (2 units per 0.5 cc of 2% suspension) are then added and the mixture is again incubated for 1 hour. Readings are recorded immediately after removal from the waterbath and after 12 hours in the icebox. It is important to titrate a serum against 3 to 4 antigens (infected, non-infected cultures of American and Australian virus strains), thus at least 48 tubes are required for the evaluation of one serum.

A recent shipment of Australian kakatoes and parrots to California offered an opportunity to test the accuracy of the complement-fixations. In quarantine, 10 of 14 parrots, which died in an emaciated state, proved to be infected with the virus of psittacosis. The remaining 31 birds were bled, then carefully autopsied and the spleen and liver tested on mice. The sera were subjected to the delicate complement-fixation test with the results shown in Table I.

The sera of the latent-infected parrots gave strong permanent and specific reactions both with Australian and American virus culture antigens. Birds free from virus either entirely failed to react to the heated antigens, gave complete fixation of 2 M.H.D. of complement in dilutions of the sera 1:1 to 1:64 or the fixation recorded immediately after removal from the waterbath faded after 12 hours in the icebox. In the light of previous experiences with the sera of

mice, guinea pigs, and other animals which had been infected or immunized, it is believed that the fixation-reactions of the sera of virus-free parrots are indicative of a past infection. It must be reserved for future studies to decide if these reactions are also indicative of immunity. The complement-fixation test in its present state does not distinguish an infection from a sterile immunity. From the standpoint of public health, parrots which specifically react in the complement-fixation must be destroyed.

10611

Effects of Fast Neutrons on Chromosomes in Mitosis.*

A. MARSHAK.† (Introduced by John H. Lawrence.)

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The neutron source was a beryllium target bombarded by deuterons accelerated to 8 million volts in the cyclotron of Lawrence and Cooksey.[1] The neutrons so obtained were collimated in a beam as described by Aebersold.[2] The biological material to be treated was placed just outside the 10 x 10 cm port at the end of the collimation apparatus at a distance of 70 cm from the target. Ionization produced by the neutrons was measured in arbitrary "n" units, and "n" unit being that amount of ionization produced by neutrons which gives the same reading on a 100 r Victoreen thimble ionization chamber as does one roentgen of X-rays.

Six-day-old seedlings of *Vicia faba* and *Pisum sativum* were mounted on an annular wooden holder and oriented so that the root tips lay in the center of the 3-inch aperture which was covered on either side by a sheet of wet filter paper and a sheet of celluloid 5.4 thousandths of an inch thick. The cotyledons and epicotyl of the seedlings lay outside the neutron beam, so that corrections for scattering may be neglected. The much smaller seedlings of *Solanum lycopersicum* were mounted between 4 sheets of wet filter paper 3½ inches in diameter lying between 2 sheets of cellophane held on a round wooden embroidery hoop 6 inches in diameter. All seedlings

* Supported by a grant from the Committee on Radiation of the National Research Council.

† Fellow of the John Simon Guggenheim Memorial Foundation.

1 Lawrence, E. O., and Cooksey, D., *Phys. Rev.*, 1936, **50**, 1131.

2 Aebersold, P. C., *Phys. Rev.*, 1939, in press.

were kept at 23°C before and after treatment. The mouse tumors were treated *in vivo* by placing the animal which was tied on a piece of cardboard so that the tumor was in the center of the neutron beam. To obtain the dose given the tumor an ionization chamber was left immediately behind the tumor during exposure. For examination of the chromosomes only the tumor tissue adjacent to the ionization chamber was used. Exposure times varied from 5 to 30 minutes.

The root tips and tumor tissue were fixed at various intervals after the neutron treatment. From these, smear preparations stained with acetocarmine were made and examined with the microscope. In *V. faba* there were 6 instances in all the anaphases examined (approximately 5,000) in which a section of a chromosome was spread out and markedly disorganized. Such chromosome disorganizations have not been observed with X-rays. Otherwise, the chromosome abnormalities observed in anaphase were qualitatively the same as those previously described for X-rays.

As with X-rays the percent normal anaphases (those showing no chromosome abnormalities) decreases to a minimum at 3 to 9 hours after irradiation and then increases again.[3] An example is given in Fig. 1, where the percent normal anaphases is plotted as a function of time after treatment of roots of *V. faba* with 20 "n". Similar curves were obtained for all the plants used after treatment with various doses of neutrons. When the logarithm of the minimum values of the time curves are plotted as a function of dose in "n" units, straight lines are obtained as shown in Fig. 2. The data from which these curves were obtained are given in Table I.

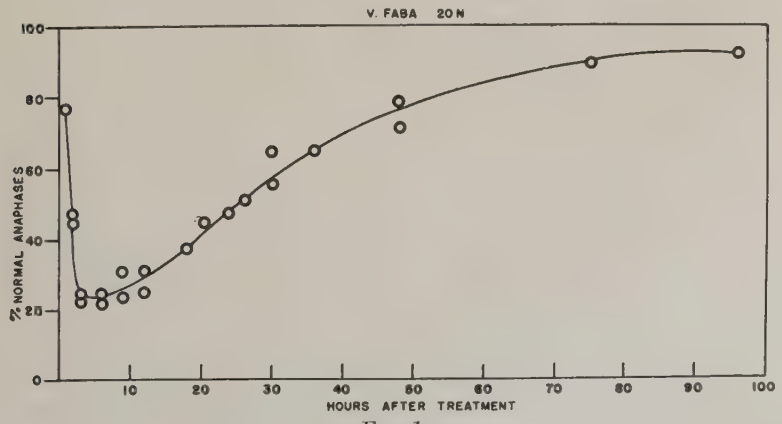

FIG. 1.

Percent normal anaphases in *Vicia faba* root tips as a function of time after treatment with 20 "n" units of neutrons.

3 Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.

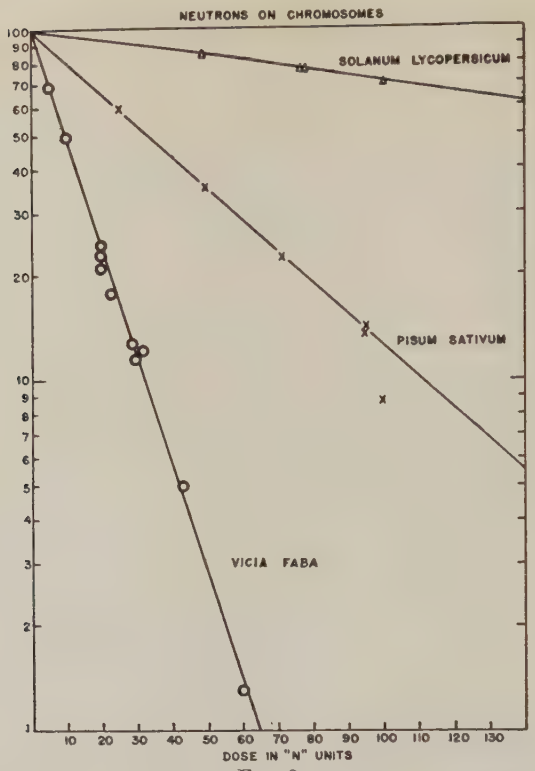

FIG. 2.

Percent normal anaphases as a function of dose of neutrons in "n" units. Ordinates percent normal anaphases at the minima of the time curves plotted on a logarithmic scale. Abscissæ, dose in "n" units. Circles represent *V. faba,* crosses *P. sativum,* triangles *S. lycopersicum.*

Three types of mouse tumors were each given 30 "n". The percent normal anaphases at 3 hours after irradiation are given in Table II.

The differences in percent normal anaphases for the 3 tumors are not considered significant in view of the possible differences in actual dose received by the tissue examined. These tumors show marked differences in sensitivity to X-rays as determined by tumor regression *in vivo* or survival of tumor fragments irradiated *in vitro.* This suggests that factors other than chromosomes may be responsible for these differences or that the loss of chromosome fragments in some types of tumors is more likely to have a lethal effect than in others.

The slopes of the survival curves of the plants and the data from the mouse tumors may be compared with the survival curves previously obtained for the chromosomes of these organisms with X-rays, as shown in Table III.

The factor of *6* obtained for the ratio of the neutron to X-ray

TABLE I.
Cells with Chromosome Abnormalities in Anaphase Three Hours After Neutron Treatment.

Dose in "n" units	Normal cells	Abnormal cells	*V. faba* Total	*V. faba* % normal
5	177	78	255	69.4
5	985	396	1381	71.2
10	189	192	381	49.6
10	473	479	952	49.7
20	25	96	121	20.6
20	122	401	523	23.4
20	138	435	573	24.2
23	64	290	354	18.0
23	17	78	95	17.9
29	31	215	246	12.6
30	21	161	182	11.5
32	16	114	130	12.3
43	2	37	39	5.1
60	2	152	154	1.3
0	1214	14	1228	99.0
			P. sativum	
25	539	364	903	59.7
50	172	314	486	35.4
71	70	239	309	22.6
95	32	242	274	14.4
95	41	255	296	13.9
100	12	124	136	8.8
0	1398	12	1410	99.0
			S. lycopersicum	
49	163	29	192	85.0
77	69	23	92	75.0
78	35	11	46	76.1
100	141	62	203	69.5
0	243	3	246	98.8

slope in all the species studied supports the hypothesis previously advanced on the basis of the X-ray data alone, that the cross-sectional area of the portion of the chromonema which is sensitive to the ionization is approximately the same size (same order of magnitude) in all of them.§ The separation of ion pairs along the track of a β

TABLE II.

Type Tumor	Normal Anaphases	Abnormal Anaphases	Total	% Normal
Sarcoma 180	417	82	499	83.6
Mammary carcinoma	304	53	357	85.1
Lymphosarcoma	1100	168	1268	86.9

§ In this connection it should be noted that neutron-X-ray ratios varying from 2 to 5 were observed when the survival of wheat seedlings, *Drosophila* eggs, fern spores, mice and mouse tumors was investigated.[4,5,6]

[4] Zirkle, R. E., Aebersold, P. C., and Dempster, E. R., *Am. J. Cancer*, 1937, **29**, 556.

[5] Zirkle, R. E., *Occasional Publications of the A.A.A.S.*, 1937, **4**, 220.

[6] Lawrence, J. H., Aebersold, P. C., and Lawrence, E. O., *Occasional Publications of the A.A.A.S.*, 1937, **4**, 215.

TABLE III.
Slopes of Survival Curves.

Species	Neutrons	X-ray	Neutron-X-ray
V. faba	70.4 x 10–3	10.7 x 10–3	6.6
P. sativum	20.5 x 10–3	3.3 x 10–3‡	6.2
M. musculus	5.4 x 10–3	9.3 x 10–4	5.8
S. lycopersicum	3.5 x 10–3	5.4 x 10–4	6.5

‡ Due to an error this slope was previously given as 4.3 x 10–3. [3]

particle from X-rays is of the order of 10^{-5} cm. With neutrons most of the ionization in tissue is along proton tracks where the separation of ion pairs is of the order of 10^{-7} cm. If there were marked differences in the cross section of the sensitive portion of the chromonema a considerable variation in the neutron-X-ray ratio was to be expected.

From the X-ray data the diameter of the sensitive portion of the chromonema was calculated to be about 10^{-7} cm on the assumption that the ion pair was the agent effective in producing chromosome abnormalities. On the same assumption neutron ionization would be expected to be less efficient than X-rays in producing abnormalities if the sensitive diameter were much greater than the average separation of ions along proton tracks. Similarly if clusters of ions rather than pairs were necessary in order to produce abnormalities, neutron ionization should be more efficient.. If the sensitive structure is of the same order of magnitude as the average distance between ion pairs of the proton track or smaller, little difference in efficiency between X-ray and neutron ionization is to be expected.

The factor 6 obtained for the neutron-X-ray ratio may be due to a greater efficiency of the neutron ionization. A similar result might be obtained if the "n" unit were 6 times larger than the roentgen. However, experiments on the physical measurement of neutron ionization conducted in this laboratory indicate that the "n" unit is not larger than the roentgen by a factor greater than 2.5 (Aebersold and Anslow, unpublished). If these measurements be accepted, the neutron ionization is about 2.5 times as efficient as the ionization produced by X-rays. There seems to be no obvious explanation for such a factor. Therefore, before reaching any definite conclusions as to whether ion pairs or ion clusters are the effective agents in producing chromosome abnormalities, it seems desirable to make a determination of the efficiency of protons which would not be dependent upon ionization chamber measurements. Such experiments are now being undertaken.

10612

Blood Coagulation During Infancy.*

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Several years ago data were published from this laboratory[1] which showed that the plasma prothrombin level in young infants is somewhat less than one-half as high as in adults. These prothrombin measurements were made with a 2-stage titration technic.[2] In the first stage, defibrinated plasma, in suitable dilution, is treated with calcium in optimal amounts, and with thromboplastin in large excess. After allowing ample time for full conversion of the prothrombin into thrombin, the latter was determined in the second stage of the titration by the addition of fibrinogen.

Since these studies were made we have undertaken further studies on infant plasma, and have discovered that in the period between the second and sixth days of life there occurs a further, though temporary, fall in prothrombin not previously recognized. We have also made a comprehensive study of infant plasma, using the one-stage prothrombin titration technic of Quick,[3] and have found that a fall also occurs with this technic. We have also observed, contrary to expectation, that except for this brief fall, the method of Quick gives results fully as high as those obtained with normal adult plasma.

This difference in results indicates the existence of important differences between the two methods. Data already published from this laboratory[4, 5] indicate the nature of this difference. It has been pointed out that the technic of Quick is one in which thromboplastin is added in large amounts to plasma. Under these conditions, thrombin is rapidly built up to the clotting level, and clotting occurs before

* Aided by a grant from the John and Mary R. Markle Foundation. Funds for two research assistants were supplied by the Graduate College, State University of Iowa. The cases used in this report were made available through the courtesy of Dr. E. D. Plass, Department of Obstetrics and Gynecology.

1 Brinkhous, K. M., Smith, H. P., and Warner, E. D., *Am. J. Med. Sci.*, 1937, **193**, 475.

2 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667; *J. Exp. Med.*, 1937, **66**, 801.

3 Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

4 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296; Proc. Soc. Exp. Biol. and Med., 1939, **40**, 197.

5 Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 595.

all of the prothrombin has been converted. The rate at which thrombin can be built up to the clotting level depends not only upon the amount of prothrombin, but also upon the specific "convertibility" of the prothrombin.[4, 5] It is our belief that in plasma of newborn infants, prothrombin conversion occurs quite rapidly, compensating for a relative deficiency in amount. The technic of Quick thus measures the summation of at least two variables—amount of prothrombin and convertibility of the latter. In our opinion this does not detract from the value of the test; on the contrary, the test measures an important summation, and under restricted conditions it gives a

TABLE I.

Case No.	Age in days	Sex	2-stage Prothrombin Test (% of normal)	Quick's Test (% of normal)	New Test (% of normal)	Whole blood clotting time (min)
1	0	M	35	110	144	—
2	0	F	36	120	134	—
3	0	F	37	70	129	4.0
4	1	F	20	110	132	—
5	1	M	37	—	123	5.5
6	1	F	44	90	122	5.0
7	1	M	23	50	76	6.0
8	1	F	29	45	60	—
9	2	M	—	—	122	—
10	2	M	53	80	104	—
11	2	M	44	115	90	—
12	2	M	22	35	41	7.5
13	2	M	20	20	28	—
14	3	F	26	50	67	5.5
15	3	F	40	—	48	—
16	3	M	23	25	34	7.5
17	3	M	20	25	28	10.0
18	3	F	22	20	25	9.0
19	4	M	28	60	63	6.5
20	4	M	37	45	56	6.0
21	4	M	—	—	55	—
22	4	M	42	60	52	—
23	4	F	23	30	43	—
24	5	M	37	—	18	—
25	5	F	27	—	93	4.5
26	5	M	40	80	97	7.5
27	5	F	—	—	117	—
28	5	M	—	—	119	—
29	6	M	6	15	32	—
30	6	M	—	90	125	—
31	6	F	31	120	139	—
32	7	F	42	—	120	5.5
33	7	F	47	130	131	—
34	8	M	39	120	104	—
35	8	F	44	100	104	—
36	8	M	—	—	138	—
37	9	F	25	100	118	—
38	10	M	41	—	139	4.5

very practical measure of the tendency to bleed. It should not, however, be assumed to give a specific measure merely of the amount of prothrombin present.

Table I gives the results obtained on 38 young infants arranged in order of their ages. Blood for these analyses was obtained from the jugular vein or from the superior longitudinal sinus,† except in case 2, where the sample was drawn from the umbilical cord. The table gives prothrombin titers obtained by the 2-stage technic; also the results obtained by the method of Quick. In the latter test, we have titrated all plasmas in the undiluted state, and also at 2- and 3-fold dilution with saline. This dilution increases the sensitivity of the method, as one could predict from the dilution curves published by Quick.[6] This dilution did not appreciably alter the results, however. In addition to these two methods we have listed the results obtained with a new clotting test which we[5] have recently devised for clinical use in cases of vitamin K deficiency. This test is similar in principle to that of Quick, but in the new test thromboplastin is added directly to whole blood instead of to plasma. This is a simplification, and as we have shown the new technic has certain theoretical advantages.

At birth Quick's test, like the new test, gives values which equal adult values, or exceed them somewhat, and this approximate equality persists even when both plasmas are diluted 2- or 3-fold with saline. In contrast to these results, the 2-stage technic shows that the prothrombin level is somewhat less than 40% of the adult value. In making the 2-stage test, we have used thromboplastin of many sources, including lung and brain of man, ox, and rabbit; also we have used cephalin, and with all of these we have obtained substantially the same result, indicating that maximum conversion had been obtained. Furthermore, we have mixed infant plasma with an equal volume of normal adult plasma, and with the 2-stage technic have obtained values half-way between the two. Nor is there any evidence of excessive antithrombic activity in the plasma of newborn infants. In fact, the plasma is diluted 20-30-fold before the test is made, and very little antithrombic activity remains. The maximum thrombin titer developed in the first stage of the titration is well maintained for several minutes. All of the evidence at hand thus indicates that the 2-stage technic gives the full titer of prothrombin, both in infants and in adults.

During the next few days of life, both Quick's test and the new

† The fontanelle punctures were kindly performed by Drs. G. W. Wagner and E. F. VanEpps.

6 Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

test show a surprising fall, levels of 30-50% being quite common. In some cases the fall is apparent as early as the second day, but in others it seems to develop later. In all cases studied on the third and fourth days the decrease was evident.

A careful survey of the data also shows a fall in prothrombin by the 2-stage technic though the fall is not so great as with other tests. We have made a special study of those cases which were less than 50% by Quick's test. In this group the 2-stage titration gave an average value which was 29% of normal, whereas in all cases above this level an average of 41% was obtained. One must conclude that the prothrombin titer by the 2-stage technic does fall, but the fall by the one-stage tests tends to be greater.

In our series, whole blood clotting time was determined in a limited number of cases. In this small series there is considerable variability, but there is some evidence that the clotting time is moderately prolonged between the third and fifth days, corresponding to the fall in prothrombin. This prolongation of clotting time was also observed by Rodda.[7] It is a curious fact that whole blood clotting time does not show much prolongation until the various prothrombin tests reach very low levels. As we have pointed out already[5] this fact suggests that thromboplastin variations may compensate for prothrombin deficiency. In the various prothrombin tests, thromboplastin is added in such large amounts that these variations are effaced, and the true prothrombin deficit makes itself evident.

At the end of 5-7 days all 3 prothrombin tests returned to approximately the values prevailing at birth. Data not given in the table show that Quick's test and the new test undergo no very significant change during the next 12 months. We have also confirmed the older observation of this laboratory[1] that the prothrombin level by the 2-stage technic rises gradually to adult levels during this 12-month period. Neither in this series, nor in the older one has sex had any definite effect on the results.

The fall in prothrombin which occurs in the first week of life was not detected in the earlier studies from this laboratory, for in those studies very few readings were taken between the second and eighth days. The fall in prothrombin is particularly significant in view of the fact that it is during this period that one sees cases of hemorrhagic disease of the newborn. Among the cases previously reported from this laboratory was a case of this disease in which the 2-stage method showed a profound lowering of the plasma prothrombin level to be the cause of the bleeding. In a study of this

[7] Rodda, F. C., *Am. J. Dis. Child.*, 1920, **19**, 269.

problem one must appreciate the fact that the convertibility of prothrombin deserves attention equally with the problem of the actual prothrombin level. By combined study with one-stage and 2-stage methods it is possible to evaluate both factors.

The cause of variation in convertibility has not been determined. In rabbit plasma the prothrombin is much more readily converted than in the plasma of man,[4] despite the fact that the actual prothrombin levels in the 2 are almost identical. We have also seen human cases in which a lowering of the prothrombin level was compensated by increased convertibility. Whether the variable convertibility represents differences in the prothrombin itself, or in the amount of "antiprothrombin" or in other factors must be answered by future research.

Summary. A study of 38 normal infants confirms previous work from this laboratory that the plasma prothrombin level is low in early infancy. It is also shown that an additional fall, not previously recognized, occurs between the second and sixth days of life. Evidence is presented to indicate that the rate of thrombin formation during coagulation depends upon convertibility of prothrombin as well as upon the amount of the latter. In newborn infants rapid convertibility of prothrombin compensates for deficient quantity of prothrombin. Evidence also suggests that variations in thromboplastin serve, in some circumstances, to compensate for a deficiency in the amount of prothrombin.

10613 P

Oxidation of Citric Acid by *Coli-aërogenes* Bacteria.

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Species of *Aërobacter* and *Citrobacter* utilize citric acid as a sole source of carbon in contrast to *Escherichia*. Previous work[1] has established the nature of the anaërobic dissimilation of citrate by *Aërobacter*. The present communication reports the results of an investigation of the aërobic breakdown of citric acid by *Aërobacter indologenes* and *Citrobacter freundii*.

1 Brewer, C. R., and Werkman, C. H., 1939, accepted for publication in *Enzymologia*.

TABLE I.
Serial Aërobic Dissimilation of Citric Acid by *Aërobacter indologenes* and *Citrobacter freundii*.
Products expressed as millimoles per 100 mM citrate fermented.

Organism	Sample No.	Time, hr	Citrate fermented, mM	H_2 mM	CO_2 mM	Formic acid mM	Acetic acid mM	Succinic acid mM	Non-reducing carbohydrate as glucose mM	O_2 uptake mM	Carbon recovery %	Redox index
A. indologenes	1	39	50	14.5	162.9	7.0	159.5	14.4	—	4.0	91.1	1.08
,, ,,	2	119	0	32.5	259.9	0	121.2	3.1	—	107.7	85.8	0.96
,, ,,	3	322	0	37.2	418.4	0	51.8	0	2.6	264.5	89.6 98.4*	0.97
C. freundii	1	39	36.8	0	194.9	0	79.2	42.0	—	69.9	86.9	0.98
,, ,,	2	202	13.2	0	516.0	0	2.2	0.3	—	352.0	86.9	1.03
,, ,,	3	322	0	0	521.7	0	1.0	0	2.2	378.7	89.5 98.7*	0.99
A. indologenes†				31.9	173.1	4.3	152.5	13.1	—	—	97.1‡	1.04

*Plus carbon in residue from ether extraction.
†Anaërobic dissimilation presented for comparison.
‡3.5 mM acetylmethylcarbinol, 3.9 mM 2,3-butylene glycol, and 6.2 mM lactic acid were produced but are not shown.

The course of citrate-oxidation was followed by serial analysis of media containing 0.1 M citrate as a sole carbon source. After inoculation, the media were kept saturated with air and the gaseous products removed continuously by forced circulation of the gas in a macrorespirometer. Results are presented in Table I.

During the first 39 hours only 4 mM O_2 were consumed by *A. indologenes* although the medium was saturated with air. By comparison with the anaërobic dissimilation shown in the bottom line of the table, the products during the initial stages of aërobic citrate breakdown appear to be those of a normal fermentation. The second and third analyses demonstrate that the action of oxygen is on acetic, succinic, and formic acids rather than citric acid itself. It is noteworthy that hydrogen was produced throughout the experiment even when its only apparent source was the aërobic dissimilation of acetic or succinic acid.

The experiment with *C. freundii* shows that in contrast to *A. indologenes* the former attacks citrate less vigorously but oxidizes the products of citric acid more completely. Hydrogen is not produced. Accumulation and subsequent oxidation of acetic and succinic acids is apparent as with *Aërobacter.*

Carbon recoveries were consistently lower in aërobic than in anaërobic experiments. The undetermined carbon was found to be non-volatile and non-ether-soluble. The properties of the unidentified material resemble those of complex carbohydrates, suggesting an oxidative assimilation of a part of the organic acids.

Respirometric experiments using the Barcroft-Warburg apparatus, on the effect of sodium azide on citrate-oxidation revealed increases in O_2 uptake and CO_2 production by cell-suspensions in the presence of M/1200 NaN_3 and by proliferating cells with M/4000 NaN_3 (Table II) (*cf.* Clifton[2, 3]). Similar effects were obtained with cell-

TABLE II.

Effect of NaN_3 on Aërobic Citrate Dissimilation by Proliferating *Aërobacter indologenes.*

200 μl citrate/flask. 41 hours.

Molarity NaN_3	CO_2 μl	O_2 μl
0	839	549
0.000125	900	608
0.0001875	970	645
0.00025	1001	701
0.000375	97	17

2 Clifton, C. E., *Enzymologia,* 1937, **4**, 246.

3 Clifton, C. E., and Logan, W. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 619.

suspensions of *A. indologenes* on *l*-malate, succinate, pyruvate, acetate, and aconitate. Aconitate was attacked aërobically only after an induction-period and anaërobically not at all. Citraconic, itaconic, tricarballylic, and α-OH isobutyric were not dissimilated either aërobically or anaërobically.

Other microrespirometric studies have shown that the respiratory quotients of oxidations of citric, aconitic, oxaloacetic, *l*-malic, fumaric, succinic, and pyruvic acids by *A. indologenes* decrease with time, indicating that the first steps in the oxidation of these acids are anaërobic.

Schemes for oxidation of citric acid by animal tissue[4] in which the citrate is oxidized stepwise through α-ketoglutaric, succinic and oxaloacetic acids cannot apply to the bacterial oxidation because the latter produces more acetate and less CO_2 in the early stages (Table I) than required by the former.

The oxidation of citric acid by *coli-aërogenes* bacteria proceeds through the normal anaërobic fermentation, to products that are dehydrogenated to CO_2, H_2O and assimilated to complex carbohydrate-like materials. The synthesis of "carbohydrate" is inhibited by NaN_3 as evidenced by increased O_2 uptake and CO_2 production.

10614 P

Some Effects of Low Choline Diets.

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The importance of choline in the prevention of "fatty livers" has been previously demonstrated by the investigations of Best[1] and of Channon.[1] The following experiments indicate that the production of a fatty liver on a low choline diet is only one manifestation of a more fundamental deficiency condition.

Male rats, 40 g in weight and 24 days of age, were used in groups of ten. The basal diet consisted of fibrin-4, casein-8, dried egg white-3, salt mixture[2]-4, calcium carbonate-1, codliver oil-5, lard-35, agar-2, and sucrose-38. The water soluble vitamins were supplied

4 Martius, C., *Z. physiol. Chem.*, 1937, **247**, 104.

1 Best, C. H., and Channon, H. J., *Biochem. J.*, 1935, **29**, 2651.

2 Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

by a daily supplement of 0.02 mg of thiamin chloride, 0.02 mg of riboflavin, 0.04 mg of nicotinic acid and 0.1 cc each of concentrated extracts of rice polish and hog liver. The rats consumed 4 to 5 g of food per day. The term fatty liver refers in every case to enlarged livers containing from 8 to 12 times the normal weight of chloroform-soluble substances.

This newly recognized effect of choline deficiency was brought to light when rats were killed and examined at the end of a 10-day experimental period. At this time 90% of the rats showed markedly hemorrhagic kidneys as well as fatty livers unless choline was added to the diet. Similar results were obtained when the vitamin supplement was omitted, when it was fed separately, when it was mixed with the basal ration and when it was doubled in amount. Although the minimum effective level has not yet been determined, the degeneration of the kidneys was prevented if the rats received 2 mg of added choline daily. This amount had no effect on the liver fat. Ten mg of choline per day were required to prevent the fatty liver. A high fat diet was not required for the production of the deficiency due to low dietary choline. The typical fatty livers and hemorrhagic kidneys occurred on a diet in which the lard was decreased from 35 to 15%. The deficiency condition was severe if all of the dietary protein was supplied by fibrin. The livers were fatty but the kidneys were normal if all of the protein consisted of casein alone or of dried egg white alone.

Other indications of a severe pathological deficiency condition were observed in addition to the gross hemorrhagic appearance and enlargement of the kidneys. The rats on the low choline diet were noticeably sick, the spleen was enlarged and the thymus was uniformly decreased to approximately one-half its normal weight. The microscopic examination of the kidney tissue showed extensive glomerular and tubular degeneration with hemorrhagic areas in the cortical region particularly.

The importance of the absolute and relative amounts of certain amino-acids in determining the choline requirement was suggested by the difference in results obtained with casein or egg white, and with fibrin. The fact that fatty livers occurred in these young choline deficient rats regardless of the protein used and the fact that much larger amounts of choline were required to prevent the fatty liver than to prevent the renal lesion indicated that certain amino-acids in casein and in the protein of egg white might have "spared" the small amount of choline in the basal ration so that none of the deficiency symptoms appeared except the fatty liver when these proteins were used.

Beeston and Channon[3] have observed an enhancing effect of cystine on fatty liver production and Tucker and Eckstein[4] have shown that methionine has the opposite effect. Furthermore, Newburgh and Curtis[5] and Cox, Smythe and Fishback[6] have reported the occurrence of hemorrhagic kidneys on a diet containing casein and added cystine and Hartwell[7] noted a similar effect on diets containing edestin. Our data suggest that these renal lesions were in reality due to choline deficiency and experiments to answer this question are now in progress.

The remarkable effect of small amounts of choline in preventing a severe pathological state associated with hemorrhagic degeneration of the kidneys demonstrated its important rôle in the maintenance of normal kidney structure in young rats. The choline requirement is dependent upon certain other factors, among which the methionine-cystine ratio of the protein may be particularly important. It is provisionally suggested that the choline requirement is increased by dietary protein relatively richer in cystine than in methionine.

10615 P

Observations Indicating Absence of Glomerular Intermittence in Normal Dogs.*

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Khanolkar[1] and Hayman and Starr[2] have reported experiments on rabbits which they interpreted as showing that usually only a fraction of the renal glomeruli are open to circulating blood. The principle in both sets of experiments was essentially the same, the injection of hemoglobin or dye into the blood stream with subsequent examination of the kidneys to determine what fraction of the capsules or glomeruli

3 Beeston, A. W., and Channon, H. J., *Biochem. J.*, 1936, **30**, 280.

4 Tucker, H. F., and Eckstein, H. C., *J. Biol. Chem.*, 1937, **121**, 479.

5 Newburgh, L. H., and Curtis, A. C., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 963.

6 Cox, G. J., Smythe, C. V., and Fishback, C. F., *J. Biol. Chem.*, 1929, **82**, 95.

7 Hartwell, G. A., *Biochem. J.*, 1928, **22**, 1212.

* This work was aided by a grant from the Commonwealth Fund.

1 Khanolkar, V. R., *J. Path. and Bact.*, 1922, **25**, 414.

2 Hayman, J. M., Jr., and Starr, Isaac, Jr., *J. Exp. Med.*, 1925, **42**, 641.

contained the injected material. The percentage of glomeruli receiving the injected material varied in different experiments from 11 to 100.

The present paper reports findings on 12 kidneys of 8 normal dogs which are interpreted as evidence that all the glomeruli are normally open all the time. The renal arteries were exposed usually by flank incision, sometimes by midline laparotomy, under nembutal, 32 mg per kilo, intravenously. Injection of 0.4 to 0.5 cc filtered Higgins Eternal Black ink into renal artery was completed in 5 seconds, renal vessels were clamped 5 seconds after end of injection. The injection was through a fine hypodermic needle, sometimes with and sometimes against the stream. The kidneys were removed soon after; 3 to 6 blocks taken from different parts of the kidney were put into 10% formalin over night and frozen sections of 100 micra cut. From 7 to 12 sections of each block were counterstained with eosin and permanently mounted. With this technic the ink-free as well as the ink-containing glomeruli are easily seen and the proportion of injected to uninjected readily determined in a very large sample. Since each section contained 200 to 800 glomeruli, with 30 to 50 sections from each kidney, there were 10,000 to 30,000 glomeruli inspected with each kidney.

In every case it was found either (a) that every glomerulus was injected or (b) that the distribution of injected and uninjected was such as to indicate that failure of injection was due to the manner of distribution of ink in the larger preglomerular vessels rather than to closure of the glomeruli themselves or of the afferent arterioles. Of 12 kidneys examined the injection was into the main renal artery in 10 cases; 2 of these showed every glomerulus injected. In 2 cases the injection was into 1 of the 2 main branches of the renal artery just outside the kidney; in these 2 all the glomeruli of one half were injected, none of the other. In 1 case the boundary was very sharp. in the other there was a boundary zone 3 or 4 mm wide within which there were both injected and uninjected, the proportion of injected in the zone increasing as one passed from the uninjected to the injected side. On the injected side the vessels other than glomeruli were also injected, but not on the uninjected side.

The remaining 8 cases are of particular interest; in all of these the injection was into the main renal artery. In 3 of these, one-half of the kidney was completely injected, the other half completely uninjected; the picture resembled the 2 cases where the injection was into one branch of the artery. In one of these 3 cases the boundary was very sharp, in the other 2 there was an intermediate zone a few

millimeters wide. In one case the plane separating injected from uninjected side passed sagittally through the pelvis, in the other 2 it differed somewhat from this plane. The only acceptable interpretation of these cases is that all of the ink by chance was swept into one of the 2 main branches of the artery; the only alternatives are that all the glomeruli in one-half of the kidney are closed while all those in the other half are open, or that one of the 2 main branches of the renal artery is showing intermittence.

In the other 5 cases there were various degrees of transition between the bilaterally symmetrical hemi-injection of the above 3 cases and a complete injection; in one case the injection was complete except for a wedge of about 1/40 the kidney volume. As would be expected, isolated fields within the various boundary zones showed a picture which, if considered alone, was quite compatible with the view of glomerular intermittence. The complete picture, however, in my opinion, gives undoubted evidence that incompleteness of injection is in all cases due to a distribution of ink in the preglomerular vessels which is determined by the characteristics of stream flow; if the picture is to be ascribed to intermittence, such intermittence must be assigned to some relatively large artery. In any area where the glomeruli are injected the vessels other than glomeruli are also injected and *vice versa*. Ink injected into the main renal artery, even though in some cases the injection was made against the stream, may by chance completely escape one of the 2 main branches or any subsequent smaller branch. It is obvious that if one depended for his conclusions upon the total count of injected glomeruli, as did Hayman and Starr, varying degrees of completeness of injection would be obtained. The procedure employed in the present paper of counterstaining the sections has made it possible to determine directly the proportion of injected to uninjected glomeruli and, more important, details of their distribution.

A few normal rabbits have also been observed, some with injection into the renal artery and some into the aorta; the conclusions to date are the same as reached above with dogs.

10616 P

The Propagation of St. Louis Encephalitis Virus in Mouse Testicle.

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The virus of St. Louis encephalitis displays so marked an affinity for nervous tissue that inoculation by any route other than the nasal or cerebral leads to infection only when relatively large amounts of virus are used. That this neurotropic tendency may be limited, however, was suggested by Webster and Clow,[1] who found that the virus apparently multiplied to some extent in the spleen.

With a view to modifying the tissue affinities of the virus, we resorted to testicular passage in the Swiss mouse. To initiate the testicular series, we used the Hubbard strain of virus, isolated in 1937.[2] A 10% suspension in broth of a mouse brain from the 68th intracerebral passage was injected in 0.03 cc amounts into the testes of 4 mice. After 5 days, 3 animals were sacrificed and the testes removed with sterile precautions by the abdominal route. Two testicles were frozen and preserved (in each passage) in order to avert loss of the testicular virus in the event of bacterial contamination or other accident. The remaining testes were weighed, ground without abrasive and suspended in sufficient broth to make a 10% emulsion. The supernatant fluid obtained after allowing gross particles to settle out by gravity constituted the inoculum, which was always cultured in broth and on blood agar plates.

The above procedure was adopted as a routine, passage being made at 5-day intervals, with groups of 4 mice, from 0.02-0.03 cc being injected into each testicle. By means of a 0.25 cc tuberculin syringe and a 27 gauge needle, this amount can be readily introduced into the testicle, although care must be exercised to avoid rupturing the organ. At each passage, 0.03 cc of the testicular suspension was injected intracerebrally into mice as a check on the presence of virus. As a precaution against possible loss of the virus, the experiments were carried on in 2 parallel series of mice.

Up to the present time, the virus has undergone 12 passages

1 Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1936, **63**, 433.

2 McCordock, H. A., Smith, M. G., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 288.

through the testicle, with no evidence of diminution in virulence as tested by the cerebral route.

We have found that the virus multiplies in the testicle with facility and in 3 days reaches a titer of 10^8 M.I.D. per testicle. The titer is approximately the same as that reached in the brain after intracerebral inoculation. This high virus content of the testicle persists for at least 2 weeks, since dilutions of testicular tissue of 10^{-6} are still infectious for mice at the end of this time; titrations at intervals longer than this have not been done as yet.

Levaditi and Lépine,[3] who compared the effects of various routes of inoculation of the St. Louis encephalitis virus in mice state that an occasional animal succumbs to encephalitis following testicular inoculation, but the majority remain unaffected and become resistant to cerebral introduction of virus. Most of our animals showed no ill effects during our observation period of 3 weeks; the occasional deaths were preceded by listlessness and ruffling of fur, and at no time did we note the occurrence of definite cerebral symptoms. Bacteriologic cultures of brain material from these mice were sterile and cerebral passage to test mice did not result in encephalitis.

10617

*The Assay of Vitamins K_1 and K_2.

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To explore more thoroughly the potencies of Vitamins K_1 and K_2, we have assayed the two pure compounds by 3 different procedures and a modification of one of them. The methods are: 1. The procedure previously described by us.[1] 2. A procedure suggested by Ansbacher's[2] work but differing from the method[3] which he finally

[3] Levaditi, C., and Lépine, P., *Les ultravirus des maladies humaines*, 1938, Librairie Maloine, Paris, V. 1, 513.

* We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

[1] Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 478.

[2] Ansbacher, S., *Science*, 1938, **88**, 221.

[3] Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

adopted. 3. A procedure very similar to that used by Almquist[4] but differing in the details of the actual determination of clotting time. 4. A procedure differing from (3) only in that the vitamin dissolved in 0.20 cc of sesame oil was administered separately each day.

The basal diet used in all of our experiments is one described by Almquist. Its composition is: fish meal, 17.5 parts; dried brewer's yeast, 7.5 parts; ground polished rice, 73 parts; sodium chloride plus small amounts of cupric and ferrous sulfates, 1.0 part; and cod liver oil 1.0 part. The fish meal and yeast were extracted with hot isopropyl ether before incorporation in the diet. Using this diet it has been found that a severe deficiency can be produced in AAA grade chicks within 2 weeks.

As previously defined our unit is that quantity of vitamin which produces a clotting time of 10 minutes or less in 50% of a group of 10 or more chicks which have been fed for 14 days immediately following receipt from the hatchery on a diet practically devoid of Vitamin K. Our experience indicates that the degree of deficiency of different lots of chicks varies considerably but that the main variation seems to be seasonal. During the late winter and early spring the deficiency is greatest. At the time we adopted 0.8 mg of our standard preparation as a unit 50% of the birds responded. At the present time the deficiency is more severe and consequently a larger dosage is required to produce a 50% response. Because of this variation in the degree of deficiency we have found it necessary to standardize our chicks with our standard extract if we are always to find approximately the same potency for the pure compounds.

As shown in our previous correspondence,[1] by means of the dosage-response curve the number of units in an unknown preparation can be determined by obtaining the percentage response of the chicks to a known amount of the preparation. A parallel assay of the standard is carried out during the same period with a group of comparable chicks. This procedure enables one to correct for any variation in deficiency of a given group of chicks.

Using the dosage-response curve and our data (No. 1) in Table I, 69% and 18% responses correspond respectively to 0.95 and 0.47 mg of our standard. Since 0.8 mg is by definition 1 unit and the response to 0.8 mg of this particular group of birds was 18%, 2 micrograms of Vitamin K_1 is equal to 0.95/0.47 or 2 units. One microgram is therefore equal to 1 unit and the potency of K_1 is 1000 units per milligram. The calculations for 2, 3 and 4 of Table I were carried out by the same procedure.

Since the speed of the 18-hour assay procedure would greatly

[4] Almquist, H. J., Mecchi, E., and Klose, A. A., *Biochem. J.*, 1938, **32**, 1897.

expedite our work, we have determined the dosage-response curve to our standard preparation. Although fewer chicks have been used in this work than were employed in the construction of the curve for the 3-day procedure it appears that the 50% response is given by about 70% of the dosage which was required to produce a 50% response in the 3-day method.† The curves are parallel but the curve for the 18-hour procedure lies to the left of the curve for the 3-day method. The data given in Table I under the 18-hour procedure were used according to the principles of the preceding paragraph in calculating our standard units per milligram for K_1 and K_2.

In view of the interesting work on Vitamin K conducted by Almquist and his collaborators it has seemed desirable to ascertain how his assays compare with ours. Chicks that had been maintained on the basal diet for 7 days after delivery from the hatchery were divided into test groups of 10 chicks (5 chicks to a cage). They were

TABLE I.

No.	Products tested	Amt of compound administered orally (micrograms)	No. of chicks (15 days of age)	Response (normal clotting time) % positive	Dosage equivalent to percentage response, mg	Our standard units per milligram
			3-Day Assay Method.			
1.	Vitamin K_1 (alfalfa)	2.0	16	69	.95	1000
	Standard prep.	800.0	16	18	.47	
2.	Vitamin K_1 (alfalfa)	1.5	9	55	.82	1000
	Standard prep.	800.0	8	25	.54	
3.	Vitamin K_2 (fish meal)	1.5	10	30	.59	660
	Standard prep.	800.0	10	30	.59	
4.	Vitamin K_2 (fish meal)	2.0	10	70	.96	770
	Standard prep.	800.0	9	33	.62	
			18-Hour Assay Method.			
5.	*a.* Vitamin K_1	0.75	8	37	.44	770
	b. "	1.00	8	50	.57	740
	Standard prep.	600.0	8	50	.57	
6.	Vitamin K_2	2.00	10	70	.77	500
	Standard prep.	800.0	10	70	.77	
7.	Vitamin K_2	2.00	20	70	.77	480
	Standard prep.	400.0	18	33	.40	

† Actually, with birds of the *same degree of deficiency* approximately twice as much vitamin is required for a 50% response by the 3-day procedure. However, this is not a factor to be considered in this discussion.

TABLE II.
Slightly Modified Almquist 7-day Curative Method.

No. of chicks	K_1 per kg of diet expressed in micrograms	Avg clotting time for each test group in minutes	No. of chicks	K_2 per kg of diet expressed in micrograms	Avg clotting time for each test group in minutes
9	40	10.1	9	80	11.2
9	80	5.8	8	160	6.4
8	160	4.6	10	240	3.2
9	160	4.6	8	320	3.0

Of 23 control chicks 3 had clotting times of 31, 32 and 45 minutes, and 20 clotting times of over 1½ hours. All of the controls except the one with clotting time of 31 minutes bled to death.

kept in a dimly lighted room and were fed on the basal diet supplemented with Vitamin K for 7 days. Groups of 10 negative controls were started on the basal diet at the same time, but usually some of these died before the experimental period, 7 days, had elapsed. We deviated from the Almquist procedure in that we followed our usual procedure in obtaining blood samples and determining the clotting time; however, the results are so consistent and clear-cut that it is not likely that any appreciable difference in results was introduced. The results are shown in Table II.

These data indicate that Vitamin K_1 is approximately twice as potent as K_2 and confirms our previous observations. During the 7-day test period 80 micrograms per kilo of diet for Vitamin K_1 and 160 micrograms per kilo for K_2 are adequate for restoring a normal clotting time. Since a chick ingests approximately 75 g of food during the 7-day period the requirement of Vitamin K_1 for each chick per day is approximately 0.9 micrograms or 1.8 micrograms of K_2.

In addition to the administration of the vitamins mixed with the food the vitamins dissolved in oil have been given daily to each chick during the 7-day test period. Under these conditions approximately 0.45 micrograms of K_1 or 0.9 micrograms of K_2 is the daily requirement for the chicks. By the use of this technic, it is certain that each chick receives a definite amount of the vitamin and moreover the possibility of loss of vitamin by decomposition is diminished.

Summary. 1. The potency of Vitamin K_1 is approximately 1000 of our units per milligram; K_2 approximately 660. 2. The 18-hour assay procedure gives satisfactory results. 3. In a slightly modified Almquist 7-day curative method, 80 micrograms per kilo of diet of K_1 and 160 micrograms per kilo of diet of K_2 are adequate.

10618

Lactogen Content of the Anterior Pituitary of Growing Rabbits and Guinea Pigs.*

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The amount of lactogenic hormone in the anterior pituitary (AP) of male and female albino rats during growth, pregnancy and lactation has been reported by Reece and Turner.[1] In a continuation of this work, similar data are being obtained on the guinea pig and rabbit. The present paper presents our observations upon the growth phase.

Normal animals were sacrificed, in increasing weight groups. The pituitaries, thyroids, adrenals and gonads then removed and weighed. The pituitaries were kept frozen until assayed by the Reece-Turner method. Each group of pituitaries was assayed with 20 pigeons. Both crop glands were used, and male groups were assayed in comparison with the female groups of the same weight.

The AP of the male New Zealand White rabbit has a low lactogen content during all stages of development, varying from 0.88 B.U. per pituitary in the 500 g group to 1.55 B.U. per gland in the 300 g group. As the AP increases in weight from 10.29 mg to 28.08 mg, there is actually a decrease in the lactogen concentration from .085 to .055 B.U. per mg pituitary tissue (Table I).

The lactogen content of the AP of the female New Zealand White rabbit is but slightly higher than that of the male in the younger groups. However, at the time of puberty with the pronounced weight increase of the ovary, there is a very conspicuous rise in the lactogen content (from 1.85 B.U. to 8.75 B.U. per AP).

The observations on guinea pigs (Table II) reveal a number of distinct species differences in comparison with either rat or rabbit. The lactogen content of the male and female in the early growth stages is quite similar but much higher than in the rabbit. They compare favorably with the concentration in the female rat AP. The most striking observation, however, is the enormous increase in the

* This study has been aided in part by a grant from the Committee on Research in Endocrinology of the National Research Council.

† Journal Series No. 612.

1 Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 266, 1937.

TABLE I.
Lactogen Content of Pituitaries from Rabbits.

Sex	No. of animals	Avg body wt, g	Avg pituitary wt, mg	B.U.* per pituitary gland (avg)	B.U. per mg pituitary tissue (avg)	B.U. per 100 g body wt (avg)	Avg wt of thyroid (1 gland) (mg)	Avg wt of adrenal (1 gland) (mg)	Avg wt of testes or ovary (mg) (1 gland)
M	20	515	10.29	0.88	.085	.17	17.75	26.98	67.58
F	16	534	10.16	1.25	.123	.23	20.98	26.64	10.62
M	20	1004	15.74	0.95	.060	.09	40.45	38.53	166.82
F	18	1068	18.58	1.79	.096	.17	40.88	36.12	15.53
M	16	1512	17.34	0.89	.051	.06	58.84	81.85	890.88
F	16	1547	23.79	1.73	.073	.11	67.23	86.65	58.84
M	20	1994	21.44	0.93	.043	.05	69.76	86.39	1391.25
F	12	1964	25.65	1.85	.072	.09	56.95	79.66	67.32
M	16	2561	24.16	1.44	.060	.06	81.59	100.63	2309.69
F	10	2542	26.47	8.75	.331	.34	72.51	100.33	129.64
M	16	3065	28.08	1.55	.055	.05	80.08	162.11	3260.94
F	6	3087	38.80	10.00	.258	.32	90.80	140.19	133.63

*Bird units.

TABLE II.
The Lactogen Content of Pituitaries from Guinea Pigs.

Sex	No. of animals	Avg body wt, g	Avg pituitary wt, mg	B.U.* per pituitary gland (avg)	B.U. per mg pituitary tissue (avg)	B.U. per 100 g body wt (avg)	Avg wt of thyroid (1 gland) (mg)	Avg wt of adrenal (1 gland) (mg)	Avg wt of testes or ovary (mg) (1 gland)
M	20	204	6.90	1.98	.286	0.97	16.00	55.60	152.45
F	20	195	6.42	1.93	.300	0.99	17.09	56.70	16.87
M	20	306	9.48	2.19	.231	0.71	19.80	67.88	417.40
F	10	299	8.37	2.31	.276	0.77	19.62	80.89	30.49
M	14	397	10.38	2.93	.282	0.74	23.92	118.75	1114.29
F	10	397	9.03	5.50	.609	1.39	27.44	110.49	24.44
M	8	499	16.21	9.50	.586	1.90	36.76	235.98	2059.38
F	9	488	13.04	6.63	.508	1.36	33.81	182.27	38.37
M	8	598	16.14	11.53	.714	1.93	36.04	204.38	2259.38
F	11	585	15.16	9.79	.645	1.67	36.05	185.24	50.18

*Bird units.

lactogen content in the male as well as the female at the time of puberty. Per gram of pituitary, the mature male and female guinea pigs have the greatest concentration of lactogen of all species so far studied when not in lactation.

Summary. While the lactogen content of the AP of the male rabbit is very low and almost constant during all phases of growth and adult life, that of the male guinea pig increases very rapidly at puberty and even exceeds the content of the mature non-lactating female. In the case of the females of both species, there is a marked rise in the lactogen content of the AP associated with increased ovarian weight at sexual maturity. In a comparison of species, on the basis of the lactogen per gram of AP, it was observed that the mature female guinea pig ranks first with about 600 B.U., the albino rat second with about 500 B.U., and the rabbit third with about 300 B.U.

10619

Inhibitory Action of Peptone on Sulfapyridine Adsorption.*

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The bacteriostatic effect of sulfapyridine on the pneumococcus *in vitro* and the inhibitory action of peptone have been shown previously.[1] The findings compare with those reported by Lockwood,[2] who studied the effect of sulfanilamide on the streptococcus under similar conditions. The effect of peptone in preventing drug-action suggested the possibility of an interference in adsorption of the drug. To test this, a study was made of the adsorption of sulfapyridine by activated carbon particles.

Varying amounts of activated carbon were added to solutions containing 10 mg of sulfapyridine and 0.85 g of sodium chloride per 100 cc. After allowing the reaction to take place for 15 minutes, the carbon was removed by filtration, and the filtrate was tested for the presence of the drug by the method described by Marshall.[3] Adsorption was found to occur, as shown in Fig. 1 (solid line).

* Aided by a grant from the Medical Research Fund of the Graduate School.

1 Hoyt, R. E., and Levine, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 465.

2 Lockwood, J. S., *J. Immun.*, 1938, **35**, 155.

3 Marshall, E. K., Jr., and Litchfield, J. T., Jr., *Science*, 1938, **88**, 85.

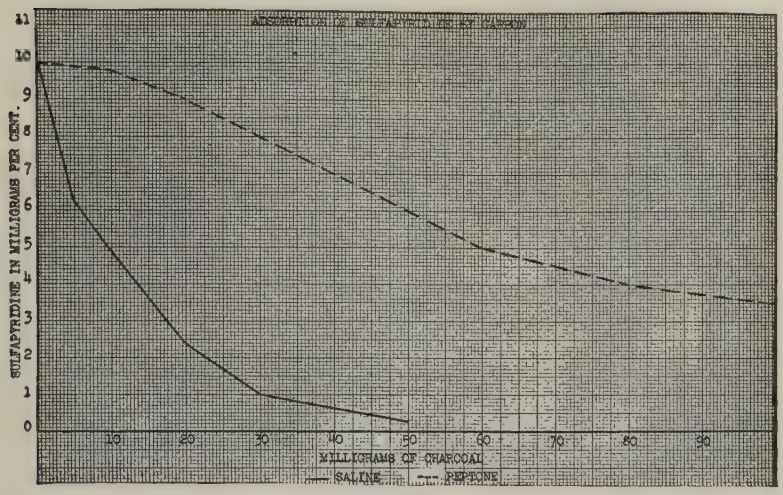

FIG. 1.

If 1% peptone (Parke-Davis) is added to the solution containing the drug, and adsorption by carbon allowed to take place, the removal of the drug is retarded. This is shown in Fig. 1 by the broken line. That this adsorption is selective is illustrated by the following experiment: 2 cc of a 1 :1000 solution of sulfapyridine were added to 8 cc of saline, and adsorbed with 20 mg of carbon. After 15 minutes, the mixture was divided into 2 parts; to one, 5 cc of 0.85% saline was added, and to the other 0.85% saline containing 1% peptone. These were allowed to stand for another 15 minutes, when they were filtered and tested for sulfapyridine. The portion to which saline had been added showed adsorption of 73% of the drug, as compared with a removal of 55% in the portion to which peptone had been added. This suggests that peptone is able to displace the drug from the surface of the carbon particles.

That adsorption occurs specifically, depending on the nature of the polar groups, has been demonstrated by the work of Jones,[4] who showed that proteins are adsorbed on special patches of collodion particles. He adsorbed 5 different proteins, and by immunological methods was able to show that none interfered with the adsorption of the others. Since peptone is composed of various amino-acids, among other constituents, it was decided to investigate the effect of individual amino-acids on the inhibition of adsorption of the drug in order to detect specific groupings responsible for this action. The acids tested, with the resulting inhibitions, are listed in Table I.

[4] Jones, F. S., *J. Exp. Med.*, 1928, **48**, 183.

TABLE I.

Amino-acid	Drug remaining (mg per 100 cc)	% inhibition
Tryptophane	5.9	50
Tyrosine	3.6	44
Phenyl-alanine	3.2	44
Alanine	1.8	None
Glycine	1.8	None
Histidine	2.0	None
Cystine	1.6	None
Proline	2.1	None
Control	1.9	—

From the above data, it is evident that the amino-acids containing aromatic groups are able to inhibit the adsorption of the drug by activated carbon. These experiments suggest the possibility that peptone, or certain of its constituents, may interfere with the adsorption of the drug on bacterial surfaces.

10620

Protective Action of Sulfapyridine Against Type II Pneumococcal Infections in Mice.*

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The remarkable results obtained by Whitby[1] in the treatment of pneumococcal mouse-infections with sulfapyridine† (M. & B. 693) have led to widespread use of this sulfanilamide derivative in experimental work. Several series of experiments, herein reported, have been conducted by a method of drug-administration quite generally neglected. This method briefly consists of mixing the drug to be studied with ground food, in any concentration selected, and allowing the mice to ingest it with their food. Hunt,[2] working in Ehrlich's Institute, also incorporated drugs in ground animal-food. The de-

* Aided by grants from the Medical Research Fund of the Graduate School of the University of Minnesota.

1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

† Merck & Co., Inc., generously supplied the sulfapyridine used in these experiments.

2 Hunt, R., personal communication to E. K. Marshall, Jr., 1939.

tails of this method will be reported later. The experience obtained indicates that mice eat a measurable amount of food during every 6-hour interval day and night. The food cups used are practically waste-proof. During the first 48 hours, the mice tend to eat increasing amounts; consequently, in the present experiments, the animals were placed on the diet plus drug 2 days before the inoculation. They were maintained on the diet plus drug for 10 days after the inoculation. Each mouse was kept in a separate cage. From daily weighings of food consumed, the drug-intake has been determined.

The organism used was a Type II pneumococcus isolated from a case of human lobar pneumonia. Its virulence has been maintained for more than a year by following the technic of Neufeld and Handel.[3] The mice were inoculated with 0.2 cc of a 1:1296 dilution of an 8-hour broth culture, subcutaneously. This represents from 4000 to 8000 average lethal doses (50% lethal dose) of the organisms. By plate count it was determined that the mice were inoculated with from 4000 to 17,000 organisms. One hundred and ninety-nine mice were used in this study. Fifty mice were placed on 0.5%, and 49 mice on 1% sulfapyridine in food, respectively. With each of these experimental groups was included a group of 50 controls. All mice used were of 20 g body-weight.

Table I shows the average drug-intake for the 2 groups of mice, in grams per kilo of body-weight per 24 hours. It can be seen that mice on diet plus 1% sulfapyridine ingest very nearly twice as much of the drug as those on diet plus 0.5% sulfapyridine.

TABLE I.
Oral Ingestion of Sulfapyridine from Food in Grams per Kg of Mouse Body-Weight per 24 hrs.

Day	0.5% in food		1.0% in food	
	No. of mice	Intake	No. of mice	Intake
1	50	0.51	49	0.95
2	50	0.98	49	1.62
	Subcutaneous inoculations with pneumococcus Type II.			
3	50	1.07	49	1.99
4	50	1.21	49	1.67
5	50	1.09	48	1.72
6	50	1.10	48	1.79
7	49	0.93	47	1.78
8	48	0.99	47	1.74
9	44	0.93	47	1.76
10	43	0.97	46	1.82
11	43	0.94	46	1.47
12	41	0.91	45	1.55

[3] Neufeld, L., and Handel, L., *Berl. klin. Woch.*, 1912, **49**, 680.

TABLE II.

Time of Survival of Mice Treated with Sulfapyridine in Food and Inoculated Subcutaneously with 4000-8000 Lethal Doses of Pneumococcus Type II.

Control	0.5% Sulfapyridine	Control	1% Sulfapyridine
	(Time of survival in hours.)		
37	86	35.5	54.2
37	109.5	35.5	84
37	135.5	35.5	153
38.2	135.5	35.5	217
43.2	135.5	35.5	230
45.5	149	35.5	237.5
46	155	35.5	251
46	199.5	36.5	251
46	204	36.5	252
48	217.5	36.5	252
48	226	36.5	252
48.5	226	36.5	252
49	248.5	36.5	252
51.5	248.5	38.7	276
51.5	248.5	38.7	276
51.5	260.5	39.5	276
53.5	260.5	39.5	276
55	261	39.5	309
55.5	264	41	
57.5	276	42.7	31 survivors
58.5	285	48	at 60 days = 63.4%
58.5	303	49.5	
60	320	52.5	
60	320	52.5	Average greater
61	327	53	than 230.5 hr
61	336.5	55.5	
64	348	57	
65.5	360	57	
65.5		59	
65.5	22 survivors	59	
65.5	at 60 days = 44%	59	
65.5		59	
65.5		59	
65.5		60.5	
65.5	Average, greater	60.7	
66.5	than 237 hr	60.7	
67.5		60.7	
68.5		61.25	
68.5		62	
69.5		62	
69.5		62	
70.5		63	
72		63.5	
72		64.7	
72		69.5	
79		73.5	
79.5		79.5	
83.5		79.5	
83.5		82.5	
92.5		83.5	
Avg		Avg	
60.1 hr		52.3 hr	

Table II shows the times of survival of all the mice which in this laboratory are observed hourly. From data thus obtained, statistical analyses can be made which show definitely the significance of the experimental observations. It is believed that observations of this type are of more value to workers in this field than previous investigators have recognized.

Summary. With subcutaneous inoculations of 4000 to 8000 average lethal doses of a Type II pneumococcus in mice, the survival rates at both 30 and 60 days were (1) with 0.5% sulfapyridine in the food, 44%, and (2) with 1.0% sulfapyridine in the food, 63.4%. It is believed that the slight variations in drug-intake from day to day are more than counterbalanced by a more or less continuous drug-absorption from ingested food plus drug.

10621 P

Character of Phospholipid (Acetone Insoluble) Fatty Acids of Serum in Infantile Eczema.*

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In 1933[1] the author observed that the total fatty acids of the serum tended to be less unsaturated in infants suffering from eczema than in control infants with clear normal skin. In the follow-up studies which were made to determine which fraction or fractions of the serum lipids were involved in this phenomenon, great difficulty was encountered in finding a satisfactory method. The success in the development of a microgravimetric technic[2] for the determination of the phospholipid fatty acids offered the opportunity to resume the study of the serum lipids presented in this communication, in which we attempted to determine whether or not the fats of this fraction were responsible for the decreased unsaturation of the serum fats in eczema.

Seven infants ranging in age from 6 to 12 months, suffering from severe, generalized eczematous eruptions of several months' duration sufficiently refractory to necessitate hospitalization, were used in this study. Care was taken to avoid the possible interference of any

* Aided by grants from Mead Johnson and Company and the Medical Graduate Research Fund of the University of Minnesota.

1 Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1198; *Am. J. Dis. Child.*, 1937, **53**, 933.

2 Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 376.

TABLE I.

Concentrations of Phospholipid (Acetone Insoluble) Fatty Acids and Total Fatty Acids in Serum with Their Respective Average Molecular Weights and Iodine Numbers in 7 Infants with Intractable, Generalized Eczema.

Case No.	Phospholipid (Acetone Insoluble) Fatty Acids			Total Fatty Acids		
	Mg %	M.W.	I.N.	Mg %	M.W.	I.N.
1	77	297	81	452	285	71
1	104	298	—	478	277	71
2	138	—	90	448	—	77
3	124	312	96	344	302	89
4	96	302	96	331	290	82
5	139	—	87	546	278	72
5	135	302	102	657	280	81
6	84	310	98	316	299	89
7	119	300	94	477	285	79
7	150	290	87	546	285	79

factors known to alter the character of the serum lipids, such as abnormalities of the dietary regimen, recent infection with fever and various therapeutic procedures, especially the topical application of ointments containing crude coal tar. Blood specimens were taken uniformly after a fast of 14 hours. The total fatty acids were determined by the microgravimetric method of Wilson and Hansen[3] and the phospholipid (acetone insoluble) fatty acids by the technic described briefly in an earlier report.[2] In all, 10 determinations were made, the results of which are presented in Table I.

It was found that the fatty acids in the phospholipid fraction of the serum in this series of infants with eczema constituted about one-fourth (range 17% to 36.6%) of the total fatty acids. The average molecular weights of these fatty acids were found to be definitely higher than those of the total fatty acids, the difference in length of the fatty acid chain being equivalent on the average to one extra carbon atom. It was further observed that the degree of unsaturation of the phospholipid fatty acids was also greater than that of the total fatty acids. In order to determine the possible significance of these findings it is necessary to consider these data in relation to the observations published recently concerning a similar study of fourteen essentially normal infants and children.[2] For the sake of brevity the average values from the previous study and those presented here are summarized in Table II.

Examination of these data reveal that the values for the total fatty acids tended to be higher in the infants with eczema than in the control group. However, in a more extensive study previously made in which the oxidative method of Bloor was used, this difference was

[3] Wilson, Wm. R., and Hansen, Arild E., *J. Biol. Chem.*, 1935-6, **112**, 457.

TABLE II.

Average Concentrations of Phospholipid (Acetone Insoluble) Fatty Acids and Total Fatty Acids in Serum with Their Respective Average Molecular Weights and Iodine Numbers in 7 Infants with Generalized, Intractable Eczema as Compared with Those in 14 Essentially Normal Infants and Children.[1]

	Phospholipid (Acetone Insoluble) Fatty Acids			Total Fatty Acids		
	Mg %	M.W.	I.N.	Mg %	M.W.	I.N.
Normal infants and children (18 determinations on 14 subjects)	107.7	303.0	112.8	350.1	288.0	107.4
Infants with eczema (10 determinations on 7 subjects)	116.6	301.4	92.3	457.5	286.8	79.0

found to have little or no statistical significance. The average molecular weights of the total fatty acids in the 2 series were the same, being 288 in one group and 286.8 in the other. On the other hand, the iodine numbers of the total fatty acids in the eczematous group were significantly lower than those of the control series. This finding has been presented and discussed previously.[1]

Concerning the phospholipid fatty acids, we find that on the average they comprised 26% of the total fatty acids in the eczema cases and 31% in the normal infants and children. It is noteworthy that the average molecular weights of the phospholipid fatty acids were essentially the same in both groups of subjects. However, they were definitely higher in the phospholipid fraction than in the other lipid fractions. As shown in Table I, the iodine number of the fatty acids of the phospholipid fraction was higher in each instance than that of the total fatty acids. From the data summarized in Table II, it is apparent that the phospholipid fatty acids of the eczematous patients were not only less unsaturated than those of the control series, but have even lower iodine numbers than the total fatty acids of the normal children.

Summary and Conclusions. In a study of the serum lipids of 7 infants with severe, generalized eczema by a microgravimetric method, the phospholipid (acetone insoluble) fatty acids were found to be more unsaturated and to have higher average molecular weights than the total fatty acids. When these findings were compared with similar information obtained in a previous study on fourteen essentially normal infants and children, the degree of unsaturation of both the total fatty acids and the phospholipid fatty acids was found to be definitely less in the eczematous subjects. These data confirm a previous finding of the author that the iodine numbers of the serum lipids tend to be lower in infants with severe eczema. In addition, they reveal the fact that the fatty acids in the phospholipid (acetone insoluble) fraction of the serum lipids partake in this reduction.

10622 P

Influence of Cultured Tissue Fragments on Sulfanilamide-Inhibition of *Beta* Streptococci.*

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It has been found that sulfanilamide causes a reduction in the size of the colony of sensitive strains of *beta* streptococci grown in tissue-culture media and that it regularly inhibits the development of the diffuse periphery usually seen around such colonies.[1]

A study has been made of the influence of tissue-fragments on these two characteristic responses of *beta* streptococci to the drug. The Maximow culture-technic was used. Details of the technic have been described previously.[2, 3, 4] A culture consists of one drop of heparinized rabbit-plasma and 3 drops of serum-extract of 7-day chick embryos, plus the required amount of sulfanilamide. To the controls a similar amount of saline was added. Sulfanilamide was sterilized by filtration. Streptococci were grown for 24 hours in rabbit-serum extract of 7-day chick embryos to which 5% rabbit erythrocytes were added. A suitable dilution of the bacterial culture made in Tyrode was added to the serum-extract.

A strain of *beta* streptococci, Lancefield group C, from the collection of Dr. F. Heilman, Mayo Clinic, was used in this study.

Cultures were incubated at 37.5°C. Observations were made at intervals during the incubation period. Measurement of colonial diameter was made in the living state (16 mm objective and 6 x ocular) using mechanical stage and ocular micrometer. Photomicrographs were made in the living state. Cultures were then fixed in Carnoy, stained with dilute Delafield's hematoxylin and mounted as whole mounts.

This strain grows rapidly in cultures without tissue and the colo-

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1 To be published.

2 King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

3 King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

4 King, J. T., Henschel, A. F., and Green, B. S., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 810.

nies show diffuse peripheries. In cultures without tissue containing 140 mg % of sulfanilamide no diffuse peripheries are seen. In cultures containing tissue-fragments even this very high concentration of sulfanilamide does not suppress the development of diffuse peripheries around those colonies within 1 mm of the fragment. Colonies more than approximately 1 mm distant from the fragment do not show this qualitative "release". The tissues studied were heart, skeletal muscle, liver, and brain. Tissue was derived from 10- to 14-day chick embryos. Heart and skeletal muscle are most effective in causing the development of diffuse peripheries in the presence of high concentrations of sulfanilamide; liver is only moderately effective and brain very slightly effective.

In spite of the fact that brain-fragments exert a very slight effect as compared with muscle in causing qualitative changes in colonies near the fragment in the presence of high concentrations of sulfanilamide, preliminary measurements show that bacteriostasis is much less in cultures containing such fragments than in cultures without fragments. In one series inhibition of colony-diameter was 12.05 and 59.34% respectively at 48 hours. Not less than 100 colonies were measured in each experimental and control set.

It has been found that when a series of cultures is planted from the same inoculated serum-extract the cultures containing tissue-fragments show higher colony-counts. It is known that sulfanilamide is less effective in the presence of a larger number of organisms. However, we know from other series and from other investigations in which this strain has been studied that this factor would be of minor importance in the observed reduction of inhibition.

Quantitative studies are in progress to determine the relative effectiveness of the 4 tissues used in antagonizing the bacteriostatic effect of sulfanilamide under various experimental conditions.

Sections of tissue-fragments cultured under these conditions show extensive cellular damage both on the periphery and in the center of the fragment.

Summary. In tissue-cultures, the characteristic effects of sulfanilamide on *beta* hemolytic streptococci are markedly inhibited by the products of tissue-breakdown.

10623

Use of Bacteriostatic Drugs in Preservation of Blood for Transfusion.

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The increasing use of blood "banks" and "stored" blood for transfusion has repeatedly brought up the problem of a suitable substance for the preservation of human blood under such conditions. Since blood is an excellent menstruum for bacteria, growth of these organisms takes place in blood even at refrigerator temperatures. That bacterial contamination occasionally occurs during the taking of blood from donors is not denied, yet the seriousness of the use of grossly contaminated blood has not been sufficiently emphasized.

Following the finding of a considerable amount of contamination among stored blood samples by routine cultural methods, it became obvious that the problem was being neglected. The risk of contamination in the periodic removal of samples for culturing purposes is too great to warrant it as a routine procedure. The possible use of

TABLE I.
Inoculation with Hemolytic Spore-forming Rod Isolated from "Stored" Blood.

	No. of colonies developing after				
	0 days	3 days	6 days	9 days	12 days
Control	56	7680	184,000	5,760,000	6,400,000
Merthiolate 1:5000	54	1280	34,600	170,000	260,000
Acriflavine 1:100,000	62	1150	179,000	9,600,000	10,200,000
Crystal violet 1:100,000	57	8	0	38,400	34,600
Brilliant green 1:100,000	51	22	200	10,000	12,800
Sulfanilamide 1:1000	61	46	33	21	39
Sulfapyridine 1:5000	53	21	0	260	210

TABLE II.
Inoculation with *Staphylococcus albus* Isolated from "Stored" Blood.

	No. of colonies developing after				
	0 days	3 days	6 days	9 days	12 days
Control	162	20,500	320,000	5,120,000	6,400,000
Merthiolate 1:5000	157	2,560	128,000	3,200,000	3,800,000
Acriflavine 1:100,000	171	1,340	20	800	960
Crystal violet 1:100,000	166	4,480	153,600	2,420,000	2,900,000
Brilliant green 1:100,000	159	310	80	7,640	9,600
Sulfanilamide 1:1000	161	33	40	63	58
Sulfapyridine 1:5000	153	158	380	270	290

TABLE III.
Inoculation with *Pseudomonas æruginosa* Isolated from "Stored" Blood.

	No. of colonies developing after				
	0 days	3 days	6 days	9 days	12 days
Control	256	12,800	522,000	24,800,000	32,000,000
Merthiolate 1:500	234	150	20	6,000	8,400
Acriflavine 1:100,000	262	146	1,900	17,800,000	21,300,000
Crystal violet 1:100,000	259	294	64,000	22,400,000	32,000,000
Brilliant green 1:100,000	238	182	7,100	16,800,000	18,800,000
Sulfanilamide 1:1000	246	144	120	210	160
Sulfapyridine 1:5000	253	153	660	3,200	2,860

a bacteriostatic substance which would be harmless by intravenous administration suggested itself.

The "selective bacteriostasis" of acridine and triphenylmethane dyes as originally demonstrated by Churchman,[1] and the bacteriostatic action of sodium-ethyl-mercurithiosalicylate (merthiolate) as pointed out by Jamieson and Powell[2] were considered. Their failure to produce the desired effect prompted the use of sulfanilamide.

The procedure consisted of inoculating 10 cc of freshly drawn citrated (0.3%) human blood with 24-hour cultures of bacteria in amounts which would introduce less than 3 bacteria per cubic millimeter of blood. The bacteriostatic substances were added and the tubes were then placed in a refrigerator at 4° to 6°C. At 3-day intervals, 0.1 cc amounts were removed from each sample, mixed with a tube of molten agar, and poured on a Petri plate. Colonies were counted after a 48-hour incubationary period.

The results with organisms most frequently isolated from contaminated blood are shown in Tables I, II and III.

The results indicate that sulfanilamide is the only consistently bacteriostatic substance in amounts compatible with intravenous dosage. Similar results were obtained with many other strains of staphylococci and other contaminants isolated from stored blood. It is not known what the ultimate effect of sulfanilamide will be on an individual receiving it; hence it cannot be stated that blood containing sulfanilamide is entirely innocuous.

1 Churchman, J. W., *Newer Knowledge of Bacteriology and Immunology*, Chicago, 1928, Chap. III, p. 19.

2 Jamieson, W. A., and Powell, H. M., *Am. J. Hyg.*, 1931, **14**, 218.

10624

Protective Antibodies against Equine Encephalomyelitis Virus in the Serum of Laboratory Workers.

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It has been shown recently[1] that a large proportion of mice and guinea pigs develop, with increasing age, physiological or structural barriers that prevent certain viruses from invading the central nervous system. This resistance is demonstrable when virus is given peripherally, as, for example, intraäbdominally or intramuscularly, but not when it is injected directly into the brain. It is not a result of prior infection nor is it associated with the presence of protective

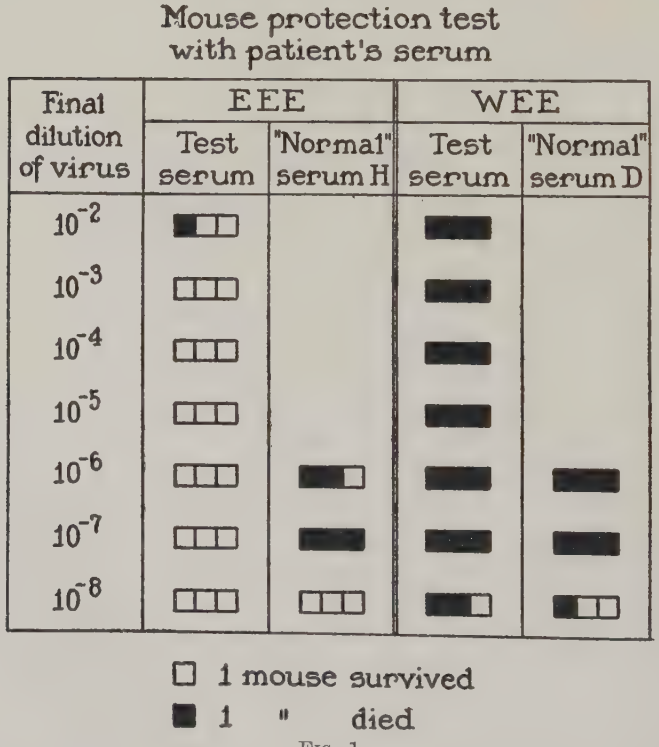

FIG. 1.

[1] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1937, **66**, 15, 35; 1938, **67**, 201, 229; Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 270; Sabin, A. B., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 595, 597.

EEE mouse protection test
with human sera

Final dilution of virus	Test sera			"Normal" serum
	A	B	C	D
10^{-3}	■□□			
10^{-4}	□□□			
10^{-5}	□□□			
10^{-6}		■■■	■■□	■■■
10^{-7}		■■■	■■□	■■■
10^{-8}		□□□	□□□	■□□
10^{-9}				□□□

Symbols as in chart 1

FIG. 2.

substance in the serum. Furthermore, in the recent epidemic of equine encephalomyelitis (E. E.) in man in southeastern Massachusetts,[2] children were predominantly affected. The older animals which resist the E. E. viruses develop systemic infection, as is evidenced by the finding of virus in the circulation and later the presence of protective antibodies in the serum. In view of this suggestive relationship of age of both the experimental animal and man to clinically apparent infection with this virus, it was thought desirable to undertake a study of the protective capacity of the serum in certain individuals in our laboratory, who had been in contact with the E. E. virus for a period extending from 1 to over 6 years. The results would indicate whether a clinically inapparent infection, as determined by the presence of protective antibody, could possibly have occurred during that time.

Serum-protection tests were carried out in mice by the intra-abdominal method of Olitsky and Harford;[3] that is, by injecting,

2 Fothergill, L. D., Dingle, J. H., Farber, S., and Connerley, M. L., *New England J. Med.*, 1938, **219**, 411; Webster, L. T., and Wright, F. H., *Science*, 1938, **88**, 305; Feemster, R. F., *Am. J. Pub. Health*, 1938, **28**, 1403; Wesselhoeft, E., Smith, E. C., and Branch, C. F., *J. Am. Med. Assn.*, 1938, **111**, 1735.

3 Olitsky, P. K., and Harford, C. G., *J. Exp. Med.*, 1938, **68**, 173.

by the intraäbdominal route, 0.03 cc of a mixture of equal parts of test serum and virus-dilutions (tenfold dilutions were used) into 15-day-old mice. Each mixture was given to groups of 3 or 4 mice. The Eastern strain of virus (E. E. E.) was derived from a stock which was frequently passaged in mouse brain and was again passaged through mouse brains immediately before use.

Two human sera, H and D, considered as "normal", were employed. The first was obtained from a worker in this laboratory in 1931 before the virus had been introduced here for study; the second was collected in January, 1938, and had been previously shown to afford no protection against E. E. E. virus. The titer of the virus in the control series, with or without these sera, was 10^{-7} or 10^{-8} (Figs. 1, 2, and 3). Another serum was added* which was obtained from a patient who 5 weeks previously had become acutely ill and had developed encephalomyelitis followed by recovery. This patient had been engaged in the procedure of inoculating E. E. virus in chick embryos. The serum, after the acute illness, protected mice against 100,000 minimal infective intraäbdominal doses of E. E. E. virus by the intraäbdominal test and showed no protection against the Western strain (W. E. E.) (Fig. 1).

Of the 6 sera collected from the individual members of our laboratory, 5 revealed no protective antibodies against E. E. E. virus (Figs. 2 and 3); variations are not significant, as shown by the con-

EEE mouse protection test with human sera

Final dilution of virus	Test sera			"Normal" serum	Broth control
	E	F	G	D	
10^{-6}	■				■
10^{-7}	■□	■□	■□	■	■
10^{-8}	■□□	■	■□□	■□	□□□
10^{-9}				■□□	□□□

Symbols as in chart 1

FIG. 3.

* We express with pleasure our deep obligation to Dr. J. H. Warvel of Indianapolis, Indiana, for this material and the history of the case.

trols. On the other hand, one (A) protected against 1,000 to 10,000 intraäbdominal lethal doses. The latter was obtained from a person who has been associated with work on E. E. virus (mostly the Eastern strain) for 6 years; at no time has he passed through an illness resembling encephalomyelitis and his general health has remained excellent. None of the 6 sera showed protective antibodies against the W. E. E. strain.

In view of the recently presented hypothesis that localized barriers develop with increasing age, or are present in particular hosts,[1] which prevent certain viruses from invading the CNS, the positive result herein reported of the presence of protective antibodies in an adult person who has been exposed to the virus in the laboratory, takes on added interest. A suggestion offered[4] is that in man, if the pattern of viral invasion from the periphery to the CNS follows that in the mouse or guinea pig, then the probability exists that in most human adults the virus may perhaps be prevented from invading the CNS by certain localized barriers. Hence adult contacts during an epidemic may have clinically inapparent infection and possibly reveal virus in the circulation. In such instances protective antibodies may be found later in the serum. Proof of this assumption would, of course, depend on further observations in the field.

10625 P

Metabolism of "Sulfapyridine-Fast" and Parent Strains of Pneumococcus Type I.

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The acquisition of "sulfapyridine-fastness" by a strain of Pneumococcus Type I has been described.[1] This induced "fastness" is associated with a fairly stable alteration in metabolism without changes in morphology, type-specificity, or virulence of the pneumococcus. The present communication deals with certain of the biochemical activities of the "drug-fast" and parent strains, together with observations concerning the action of sulfapyridine on the pneumococcus.

On the usual culture media, the drug-fast strain grows as well as the parent strain and ferments the same sugars.

[4] Sabin, A. B., personal communication.

[1] MacLeod, C. M., and Daddi, G., PROC. SOC. EXP. BIOL. AND MED., in press.

Hydrogen peroxide is formed in cultures of pneumococcus as a product of aërobic metabolism.[2] Its presence can be detected in cultures by the addition of benzidine and peroxidase; the latter may be supplied in the form of blood or potato.[3]

When grown in a shallow layer of broth exposed to air the parent strain produces an abundance of hydrogen peroxide, while little or no peroxide is formed by the drug-fast strain under the same conditions.

Penfold[4] employed blood-agar plates containing benzidine for the detection of microörganisms which produce peroxide during growth. This method has proved very useful in differentiating the two strains of pneumococcus. The colonies of the parent strain on this medium are jet black after 16-24 hours' incubation due to the production of a relatively large amount of peroxide, whereas the colonies of the drug-fast strain show only moderate browning.

Dehydrogenase-activity of the two strains. The dehydrogenase-activity of both strains was studied by determining the ability of cell suspensions to reduce methylene blue in the presence of various substrates.

Cultures of both strains of pneumococcus, grown for 9-12 hours in

TABLE I.

Dehydrogenase-activity of Parent and "Sulfapyridine-fast" Strains on Various Substrates.*

Substrate (Final concentration)	Reduction of methylene blue after 1 hour at 37°C	
	"Sulfapyridine-fast" strain SV-I/P	Parent strain SV-I
Glucose M/140	++++†	++++
Glycerol M/80	—	++++
Sodium lactate M/80	—	+++
" pyruvate M/80	—	++++
Acetaldehyde M/80	++++	++++
Sodium succinate M/80	—	—
" formate M/80	—	—
" acetate M/80	—	—
0	—	—

*Each tube contained 0.5 cc of 0.002 M methylene blue in M/20 phosphate buffer pH 7.6; 0.1 cc of plain broth as a source of coenzymes; 0.5 cc of the appropriate substrate; 1.0 cc of the suspension of pneumococci. The final volume was brought to 4.0 cc in each case by the addition of M/20 phosphate buffer, pH 7.6. The tubes were sealed with a layer of vaseline and incubated at 37°C.

†++++ indicates complete reduction; — indicates no reduction of the methylene blue.

[2] McLeod, J. W., and Gordon, J., *J. Path. and Bact.*, 1922, **25**, 139; *Biochem. J.* (London), 1922, **16**, 499.

[3] Avery, O. T., and Morgan, H. J., *J. Exp. Med.*, 1924, **39**, 275.

[4] Penfold, W. J., *Med. J. Australia*, 1922, **2**, 120.

plain broth under vaseline seal, were centrifuged under seal and the bacteria resuspended in one-twentieth volume of M/20 phosphate buffer at pH 7.6. When these precautions are observed the cell suspensions retain their dehydrogenase-activity longer than if exposed to air throughout the various manipulations.[5] Cell suspensions of this sort have been termed "resting bacteria".[6,7] To eliminate as far as possible the complicating factors associated with cell multiplication, the conditions were so arranged that reduction of the dye occurred within a 1- to 2-hour period of observation. The dehydrogenase-activity of the 2 strains of pneumococcus on a number of substrates is shown in Table I.

Of the substrates tested, glucose is an active hydrogen donator in the presence of both strains of pneumococcus and no difference in the time required for reduction of the methylene blue is observed. On the other hand, the drug-fast strain shows little dehydrogenase-activity for glycerol, lactate, or pyruvate, whereas the parent strain dehydrogenates these substrates actively. It appears, therefore, that these 2 strains of pneumococcus exhibit distinct differences in their ability to dehydrogenate certain 3-carbon compounds, namely, glycerol, lactate, and pyruvate.

In the preceding experiments the dehydrogenase-activity of the cell suspensions was tested in the absence of sulfapyridine. In the following experiments sulfapyridine was added to the reacting systems

TABLE II.

Effect of Sulfapyridine on Dehydrogenase-activity of Parent and "Sulfapyridine-fast" Strains.*

Substrate (Final concentration)	Sulfapyridine†	Reduction of methylene blue after 1 hour at 37°C	
		"Sulfapyridine-fast" strain SV-I/P	Parent strain SV-I
Glucose M/140	0	++++	++++
"	1:8,000	++++	++++
Glycerol M/80	0	—	++++
"	1:8,000	—	+
Sodium lactate M/80	0	—	+++
"	1:8,000	—	—
Sodium pyruvate M/80	0	—	++++
"	1:8,000	—	+
0	0	—	—
0	1:8,000	—	—

*System used was the same as described in Table I.

†1.0 cc of a neutral solution of sulfapyridine 1:2,000 added as indicated, making a final concentration of 1:8,000.

5 Bach, D., and Lambert, J., *Bull. Assn. Diplomes Microb. fac. Pharm. de Nancy*, 1937, **15**, 25.

6 Quastel, J. H., and Whetham, M. D., *Biochem. J.* (London), 1925, **19**, 520.

7 Cook, R. P., and Stephenson, M., *Biochem. J.* (London), 1928, **22**, 1368.

in order to determine the direct effect of the drug upon the dehydrogenase activity of resting cells of the parent and drug-fast strains. These results are shown in Table II.

Sulfapyridine, in a final concentration of 1:8,000 in the reacting system, does not inhibit the glucose dehydrogenase of either strain. However, the same concentration of the drug greatly inhibits the dehydrogenase-activity of the parent cells for glycerol, lactate, and pyruvate.

The relation of peroxide formation to the carbohydrate metabolism of pneumococcus is not entirely clear. However, suspensions of the parent cells incubated in a shallow layer in the presence of glycerol and coenzyme produce much more peroxide than if the glycerol is replaced by an equal concentration of glucose, indicating that hydrogen peroxide is produced during the metabolism of glycerol.

Summary. The acquisition of "sulfapyridine-fastness" by a strain of pneumococcus Type I is associated with a marked diminution in the production of hydrogen peroxide in cultures of this strain. Cell suspensions of the parent and drug-fast strains dehydrogenate glucose equally well. On the other hand, the acquisition of sulfapyridine-fastness is associated with a marked loss of dehydrogenase-activity for certain 3-carbon compounds (glycerol, lactate, and pyruvate). When sulfapyridine is added directly to the reacting system the dehydrogenase-activity of the parent cells for the same 3-carbon compounds is likewise much decreased.

10626 P

Specific Absorption of Antibody with Extracts Containing the Rabbit Papilloma Virus (Shope).

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The papilloma virus, or a substance that has proved inseparable from it, reacts with specific immune sera to fix complement, as previous studies have shown;[1] and tests made recently with more than a score of such sera have borne out a finding already obtained, namely that the virus-neutralizing ability of any serum is directly proportional to its complement-fixing capacity. The present work was

[1] Kidd, John G., *J. Exp. Med.*, 1938, **68**, 703, 725, 737.

undertaken to learn whether the virus-neutralizing and complement-fixing antibodies can be absorbed from the immune sera; and, if so, whether they are absorbed together, and what is responsible for their removal.

A number of immune sera have been used, some obtained from cottontail rabbits with naturally-occurring growths and others from wild and domestic rabbits with experimental papillomatosis. Various dilutions of the sera were made with saline, from 1:2 to 1:64 or higher, and these were mixed in equal parts with clear, saline extracts of the virus-induced growths, either as such or after Berkefeld filtration, in dilutions up to 1:640. The mixtures were put into the water-bath at 37°C for 2 hours, then kept overnight in the refrigerator. The relative amount and character of the specific visible flocculation, which was regularly present in the tubes containing antigen and antibody in optimal proportions, were then recorded, and the mixtures were spun at 4400 rpm for 20 minutes in an International centrifuge with 51°-angle head. The supernatant fluids thus obtained were now tested for content of virus, complement-fixing antigen and antibody, by means of standard pathogenicity, neutralization, and complement-fixation tests which have been already described.[2]

In every one of 7 experiments, in which extracts containing the virus were mixed with immune sera in optimal proportions, tests of the sort described showed that absorption of antibody had occurred; and the complement-fixing and virus-neutralizing capacities of the sera were always proportionately reduced. After the mixtures had been centrifugalized, the supernatant fluids were regularly found to be neutral when optimal or near optimal proportions of antigen and antibody had been used; that is to say, they contained no detectable amounts of virus, antigen, or antibody, as manifested by the *in vivo* and *in vitro* tests. When an excess of antigen or antibody had been used, on the other hand, a proportionate excess of the one or the other remained in the supernatant fluids after absorption and centrifugation; and whenever there was a large excess of either no visible flocculation took place in the mixtures. It was observed that a given amount of antibody could be absorbed completely by very much less of a virus-filtrate than it would neutralize, and that the amount of a virus-filtrate required to absorb antibody was even less than that required to fix 2 units of complement.

The findings thus far indicate that the virus itself is involved in the antibody-absorption. Filtered extracts of the naturally-occurring

[2] Kidd, John G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64**, 63, 79; Kidd, John G., *J. Exp. Med.*, 1938, **68**, 703, 725, 737.

papillomas of some cottontails regularly contained much of the virus, and these always absorbed antibody readily and in great amount. Extracts or filtrates of the virus-induced papillomas of other cottontails, which contained much less of the virus as indicated by their infective titer, had much less power to absorb antibody; and still others, made from the experimental growths of cottontail and domestic rabbits, which yielded no pathogenic virus, failed completely to absorb antibody in the tests, even when large amounts were used repeatedly. Extracts of the Brown-Pearce tumor, tested concurrently, had no power to absorb the antibody.

When a potent virus filtrate was spun at 30,000 rpm for 60 minutes in the air-driven centrifuge, the supernatant fluid—which contained practically none of the virus, but much protein as determined roughly by the sulphosalicylic acid test—proved devoid of capacity to absorb antibody; whereas a suspension of the pellet of sediment in the original volume of saline—which contained little protein in comparison with the supernatant fluid, but almost as much virus as the whole filtrate—absorbed antibody quite as well as the latter.

A centrifugalized virus filtrate that absorbed much antibody when used unheated or after heating at 56°C for 30 minutes, failed to do so after it had been heated at 66°C for 30 minutes, a procedure that abolished the capacity of the filtrate to fix complement and rendered it completely non-infectious.

The findings make it plain that the complement-fixing and virus-neutralizing antibodies can be absorbed together, and readily, from the sera of rabbits bearing virus-induced papillomas, when these are mixed with extracts or filtrates containing the papilloma virus; and they indicate that union of the antibody with the virus itself, or an integral part of it, is responsible for the absorption. The significance of these results, notably in relation to the findings of Salaman in similar absorption experiments with the elementary bodies and soluble antigens of vaccinia,[3] will be discussed later when the facts are reported in detail.

[3] Salaman, M. H., *Brit. J. Exp. Path.*, 1937, **18**, 245; 1938, **19**, 192.

10627 P

Temperature Changes in Skin and Muscle of Lower Extremities Following Intravenous Injections of Typhoid Vaccine.*

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(Introduced by G. Shwartzman.)

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Intravenous injections of typhoid vaccine have been used extensively in the treatment of peripheral vascular diseases.[1-4] Since our previous studies showed that the temperature of the skin and muscles of the lower extremities is not altered in a parallel manner by certain other procedures, a study of the effect produced by typhoid vaccine on these 2 structures seemed desirable. This study was limited to the changes noted in the calf muscles and skin of the feet. The range of fever employed was from 99 to 101°F, since higher temperatures are usually avoided in the treatment of peripheral vascular diseases.

The initial intravenous dose of typhoid vaccine was five million microörganisms. In some individuals this amount was inadequate to produce any reaction and it was progressively increased until the systemic temperature was raised 2 or 3°F. The temperatures of the skin surface and calf muscles were observed independently. Thermocouple needles were inserted into the calf muscles and preliminary temperature stabilization was obtained in each case. All skin surface temperature readings were made on the plantar surface of the distal phalanx of the great toe. The skin surface and calf muscle temperatures were recorded at 10-minute intervals for a period of 2½ to 5 hours after the reaction. The rectal temperature was obtained by means of an automatic registering resistance rectal thermometer. The reaction usually occurred from 60 to 90 minutes after the injection. Most of the patients complained of feeling a little cooler for a period of from 15 to 20 minutes but had no marked chills. In some the chills and tremors were violent.

* Aided by grants from the Council on Physical Therapy of the American Medical Association.

1 Goodman, C., and Gottesman, J., *N. Y. Med. J.*, 1923, **117**, 774.

2 Brown, G. E., Allen, E. V., and Mahorner, H. R., *Thrombo-Angiitis Obliterans*, W. B. Saunders Co., Philadelphia, 1928, p. 143.

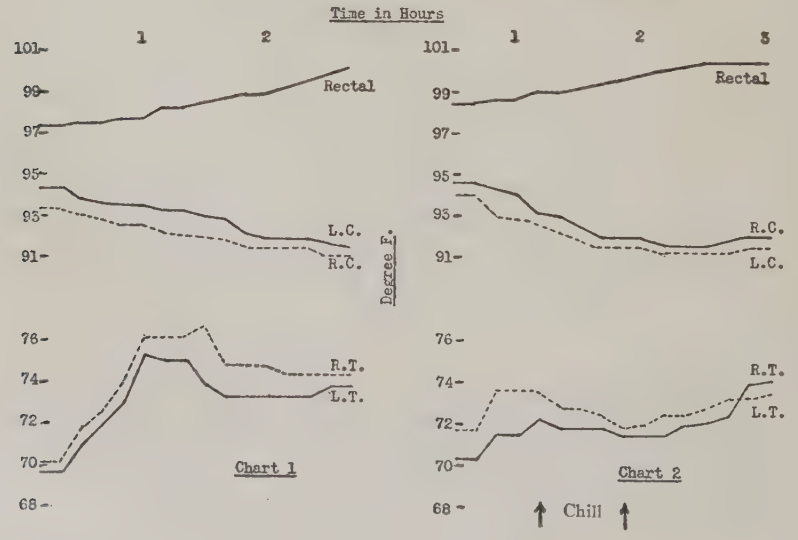
3 DeTakats, G., and Mackensie, W. D., *Surg., Gyn. and Obst.*, 1934, **58**, 655.

4 Wright, Irving, S., *M. Clinic North America*, 1934, **17**, 1429.

Twenty-eight cases were studied in this manner. In the majority of cases a rise in systemic temperature began about an hour after the injection. The body temperature continued to rise gradually and was accompanied by a gradual fall in calf muscle temperature. The chill period usually occurred about 1½ hours after the injection. The rise in systemic temperature and fall in muscle temperature continued throughout this period. The rate of change did not vary regardless of whether or not muscle tremors took place. Following the chill, the gradual rise in body temperature and fall in muscle temperature continued. In some cases the skin surface temperature rose before the occurrence of the chill. In almost all cases it dropped at the onset of the chill and rose above the initial level after the tremors subsided.

To illustrate the fact that the reactions are essentially similar regardless of whether chills and tremors occur or not, 2 typical fever charts are shown.

The data obtained in these experiments permit certain conclusions. It is noted that during fever elicited by the intravenous injection of typhoid vaccine there occurs a gradual fall in the calf muscle temperature. The skin surface temperature increases. Differences in the temperature of tissues are indicative of variations in the blood supply to those structures. It would, therefore, appear that an elevation of the systemic temperature to 101°F is associated with an increase in the circulation of the skin of the feet and a decrease in the circulation of the calf muscles. Further investigations are being



carried on to determine the changes which occur in muscles of other parts of the body, of the effects produced by temperatures exceeding 101°F, and the changes which occur later than 5 hours after the reaction. It is also important to determine whether similar alterations develop in fevers of other types.

Since the calf muscle temperature was always several degrees lower than the systemic temperature and since it actually decreased during the production of fever, it appears that the increased body temperature under the conditions described cannot be attributed to any activity in the calf muscles. This was true in spite of evidence of markedly increased muscular activity during the chill period.

Since the circulation of the skin is improved during the reaction following the intravenous injection of typhoid vaccine, such injections may be beneficial in the treatment of ulcers. However, since the calf muscle circulation is not increased, the use of this method of treatment for relief of intermittent claudication appears to have no physiological basis.

10628

Reversibility of the *Alpha* and *Beta* Phases of *Salmonella typhi*.*

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By the cultivation of *Salmonella typhi* in broth containing antiserum derived from the specific phase of *Salmonella muenchen*, Kauffmann[1] was able to isolate a variant which possessed altered flagellar antigens and which was no longer agglutinable in *S. muenchen* antiserum. The variant was obtained from only one culture and was not reversible. It was designated as a *beta* phase of the theretofore supposedly monophasic typhoid bacillus. Through cultivation in immune serums, *beta* phases were also obtained by Kauffmann and Tesdal[2] from *Salmonella schleissheim* and by Gard[3] from *Salmonella abortus-canis*. None of these induced phases were reverted to the

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

1 Kauffmann, F., *Z. f. Hyg.*, 1936, **119**, 103.

2 Kauffmann, F., and Tesdal, M., *Z. f. Hyg.*, 1937, **120**, 168.

3 Gard, S., *Z. f. Hyg.*, 1938, **121**, 139.

naturally occurring phases of the bacilli and it is questionable whether they represented phase-variation as it is observed in naturally diphasic species, or whether they were mutants produced by exposure to antiserums.

It was found by Bruner and Edwards[4] that the technic of Wassén[5] was very effective in the isolation of suppressed specific phases in the so-called monophasic nonspecific *Salmonella* types. Therefore 7 cultures of *S. typhi* in our possession were examined by this method. The organisms were cultivated in agglutination-tubes containing semi-solid agar to which had been added sufficient *S. muenchen* antiserum to confine the growth of the normal, or *alpha,* phase of the bacilli to the line of inoculation. The medium was inoculated by stabbing at one side of the tube. Outgrowths from this line represented variation in the flagellar antigens of the bacilli. By this method *beta* phases similar to the one described by Kauffmann[1] were obtained from all the cultures on the first trial. Subsequently, the experiment was twice repeated with the same results.

The variants were transferred several times in semi-solid agar containing *S. muenchen* antiserum to free them of the *alpha* phase. They were then examined by agglutination and agglutinin-absorption to insure their purity and identity, after which they were placed in semi-solid agar containing serum derived from a *beta* phase of the typhoid bacillus in an effort to revert them. Two *beta* phases derived from each of the 7 cultures at different times were tested for reversion. The only reversion noted was in one *beta* phase of the Watson strain. Under the influence of suitable antiserums this culture was highly variable and could be transformed from one phase to the other at will. No reversion was noted in the absence of antiserum, indicating that the variations observed were not due to impurity of the *beta* phase.

To further establish the reversibility of the *beta* phase of the Watson strain, it was plated in semi-solid agar and well isolated colonies fished to agar-slants and to semi-solid agar containing antiserum derived from the *beta* phase of *S. typhi.* Of 65 colonies thus examined, 2 yielded the *alpha* phase when placed in contact with *S. typhi beta* serum. The agar-slant cultures inoculated from the same colonies were pure *beta* phases. It is quite evident, therefore, that certain *beta* phases derived from *S. typhi* are reversible when placed in contact with the corresponding antiserum. The phases thus reverted are typical *alpha* phases and differ in no way from the parent strain from which they were originally derived.

[4] Bruner, D. W., and Edwards, P. R., *J. Bact.,* 1939, **37**, 365.

[5] Wassén, A., *Bull. mensuel de l'Office Internatl. d'Hyg. publ.,* 1935, **27**, 1.

10629 P

Action of Urea Upon Hemoglobin. Spectrophotometric Study of Progress of a Protein Denaturation.

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When NaOH is added to solutions of hemoglobin, a reaction is initiated whose progress may be followed very accurately by means of spectrophotometry. The changes which occur may, for convenience, be divided into 2 successive main reactions, and may be represented as follows:

1. Ferrohemoglobin (HbO_2) + NaOH → Globinoferriporphyrin (oxidized, denatured globin hemochromogen).
2. Globinoferriporphyrin + NaOH → Ferriporphyrin hydroxide (alkaline hematin) + denatured globin.

Under the conditions which have been used the reactions are unidirectional, but the addition of a reducing agent, $Na_2S_2O_4$, shifts reaction 2 in favor of the hemochromogen, as follows:

3. Ferriporphyrin hydroxide + denatured globin + $Na_2S_2O_4$ → Globinoferroporphyrin (reduced, denatured globin hemochromogen).

When $Na_2S_2O_4$ is added to globinoferriporphyrin (reaction 1), globinoferroporphyrin also is produced. The terminology which is employed is unambiguous, and has been proposed by the writer.[1] The protein component of the hemoglobin is probably irreversibly denatured in this process. It has already been pointed out by Drabkin and Austin[2] that the first reaction takes time and is the basis for the von Krüger reaction.[3]

It has been found by the writer that essentially similar reactions to the above occur when hemoglobin is denatured by reagents other than alkali—notably urea and acetamide in high molar concentrations (4 to 6 M), and HCl. In the case of the latter reagent, denaturation results in the production of the familiar acid hematin and even with fairly strong acid (0.1M) it is well recognized that the reaction takes time. During the course of this reaction the writer has found that, if enough alkali is added to just dissolve the material precipitated at the iso-electric point as the acidified solution is neu-

1 Drabkin, D. L., *J. Biol. Chem.*, 1938, **123**, xxi.

2 Drabkin, D. L., and Austin, J. H., *J. Biol. Chem.*, 1935-36, **112**, 89.

3 von Krüger, F., *Z. ges. exp. Med.*, 1926, **53**, 233; Haurowitz, F., *Z. physiol. Chem.*, 1929, **183**, 78.

tralized, the presence of the typical globinoferroporphyrin (the reduced hemochromogen) is disclosed upon the addition of $Na_2S_2O_4$.

In studying denaturation reaction rates conditions were chosen to provide for a very slow reaction with alkali. The concentration of hemoglobin (HbO_2) in the final solution was 0.1 mM per liter (where 1 mole is equivalent to 1 mole of iron porphyrin), and the concentration of total alkali in the final solution was 0.008M. The alkali used was a mixture of 0.005M NaOH and 0.003M NH_4OH. The ammonia was included so as to more than compensate for the slight ammonia production which occurred in the experiments in which 6M urea was used with NaOH added to a concentration of 0.005M. The determinations were upon solutions in closed 1 cm cuvettes at a temperature of approximately 20°C. Under these conditions the time required for one-half completion of reaction 1 was 13 hours for horse hemoglobin (HbO_2) with alkali alone added, as above. For dog hemoglobin, under the same conditions, the reaction was somewhat more rapid.

Carbonmonoxide hemoglobin of both species is appreciably more stable to alkali and to other denaturing reagents than is oxyhemoglobin. The rate of denaturation of oxyhemoglobin in the presence of 6M urea alone is approximately of the same order of magnitude as with the 0.008M concentration of alkali, when judged by the criterion of globinoferriporphyrin formation. The rate of reaction is decreased by lowering the concentration of urea to 4M, and is appreciably increased at a temperature of 38°C instead of 20°C.

The present experiments are not inconsistent with Steinhardt's finding[4] that with horse carbonmonoxy hemoglobin in 4M urea, disaggregation of the molecules of the protein into units of one-half the original molecular weight takes place independently of the process of denaturation. According to Wu and Yang[5] dog hemoglobin, in contrast with horse hemoglobin, is not disaggregated in the presence of urea. Both horse and dog hemoglobin, however, undergo denaturation in urea, and at about the same rate in the writer's experiments. From the standpoint of the possible rôle of urea in protein denaturation the following finding of the writer is of great interest: In the presence of 6M urea and 0.005M NaOH, both horse and dog oxyhemoglobin are denatured approximately 60 times as rapidly (13 minutes for one-half completion of reaction 1) as with 6M urea alone or with a total of 0.008M concentration of alkali alone, as described above. The exposure of sulfhydryl groups in the protein in

[4] Steinhardt, J., *J. Biol. Chem.*, 1938, **123**, 543.

[5] Wu, H., and Yang, E. F., *Chinese J. Physiol.*, 1932, **6**, 51.

the presence of urea[6] does not appear to account for the above finding since disaggregation in acetamide is unaccompanied by exposure of sulfhydryl groups,[6] and denaturation of hemoglobin also occurs in the presence of the latter amide.

10630 P

Prothrombin Concentration in Newborn.

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The concentration of prothrombin in the blood of babies 3 to 7 days old has been found to be essentially the same as in adult blood.[1] Curiously, however, a profound fluctuation has been observed to occur during the first 48 hours of life, as shown in Table I.

It should be noted that the prothrombin level of babies 6 hours old is relatively high and not strikingly different from cord blood. At the end of 24 hours, however, it may drop to an exceedingly low level as shown by babies 5 and 6. After 48 hours the prothrombin concentration usually has begun to return to normal. In one baby

TABLE I.

			Clotting Time, Quick's Prothrombin Method		
		Age	Undiluted plasma	Diluted plasma, 50%	Prothrombin Concentration, %
Cord blood	1		13	18	71
	2		13	18½	67
	3		13	18½	67
Baby	1	6 hr	12	17	80
	2	6 "	12½	17½	75
	3	6 "	12	18	71
	4	1 day	12½	17½	75
	5	1 "	53	105	7
	6	1 "	55	—	7
	7	2 days	13	18½	67
	8	2 "	13½	21	54
	9	2 "	17	30	35
	10	2½ days	29	47	16
		3½ "	30	48	15½
		5½ "	13	17½	75

6 Greenstein, J. P., *J. Biol. Chem.*, 1938, **125**, 501; 1939, **128**, 233.

1 Quick, Armand J., and Grossman, Arthur M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 647.

(No. 10) the return was definitely delayed since 5½ days elapsed before the concentration had reached 75% of normal.

These results offer a solution to the perplexing problem of the hemorrhagic disease of the newborn. Apparently no reserve of prothrombin is built up in the fetus, and in the first 24 hours of life this clotting factor may be reduced to a distinctly hemorrhagic level. Were it not for the prompt restoration of the prothrombin, many babies would bleed. Any delay in this recovery will naturally give rise to a bleeding diathesis, and this is very probably the cause of the hemorrhagic disease of the newborn. Potentially all newborns are in jeopardy of hemorrhage, and this undoubtedly accounts for the insistence of the mosaic law that circumcision be not performed before the eighth day.

The fact that the hemorrhagic disease of the newborn responds promptly to vitamin K therapy, as Waddell, *et al.*,[2] have reported, indicates definitely that a deficiency of this factor is the basic cause. The question remains: why the abrupt recovery? The small amount of food taken during the first 48 hours obviously can furnish little vitamin K. A new source must become available. It is well known that vitamin K is produced by bacterial action, and Greaves[3] has demonstrated that rats fed a vitamin K-free diet excrete significant amounts of the substance in the feces. Logically it follows that as soon as the baby ingests food or water, it infects its intestinal contents, which at birth are sterile. With the establishment of an intestinal flora the synthesis of vitamin K begins, which with the aid of bile is absorbed, thus ending the danger of bleeding due to prothrombin deficiency.

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[2] Waddell, W. W., Jr., Guerry, D., Bray, W. E., and Kelley, O. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 432.

[3] Greaves, Joseph D., *Am. J. Physiol.*, 1939, **125**, 429.

10631

Induction of Singing in Female Canaries by Injections of Male Hormone.*

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From the Bureau of Biological Research, Rutgers University, New Brunswick, N. J.

It is generally recognized that singing in canaries is limited to the male and as such this may be considered as a secondary sexual characteristic. In order to determine if this behavior is conditioned by secretions of the testis, male hormone was injected into female birds.

The canaries used were raised by a local breeder, Mr. J. Frederichs, who has developed a hardy strain of birds by acclimating them to life out of doors throughout the year. They were brought into the laboratory in January, a few weeks before the normal breeding season. Females can be distinguished from males during the breeding season by observing differences in the cloacal eminence. The male cloacal eminence is long, pointed and projects ventrally while that of the female is broad and is directed posteriorly. At no time did the females sing previous to injections; they gave only the characteristic "chirp" of the female and were busy carrying bits of straw and paper, to build nests.

Testosterone propionate (Oreton)† in 0.2 cc doses equal to 5 mg of hormone, was injected into the breast muscles every 3 or 4 days until singing occurred. Five birds were used in this study.

In 4 out of 5 birds, the typical male song was sung by the injected females. One of them began singing after 2 injections, the others after 4 injections. The singing was continued for periods of 5 to 13 days after the last injection, depending on how much hormone was given. The remaining bird, though it sang no sustained song, behaved like the normal males or the stimulated females in her attempts to sing. She strutted on her perch, swelled out her throat and moved it as if singing but the sounds emitted were interrupted and resembled only portions of the male song in variation and duration. This behavior subsided on the withdrawal of the hormone. On the basis of behavior, this result may be considered positive because the usual female "chirping" was abnormal in the beginning.

* Aided by a grant from the American Philosophical Society, Penrose Fund.

† The male hormone, Oreton, was furnished through the kindness of Dr. W. R. Bond of the Schering Corporation.

Apparently there was no permanent deleterious effect on the reproductive system following the injections because 3 of the 5 birds were mated and have built nests and laid eggs.

In summary, it is seen that female canaries can be made to sing the typical male song by injections of male hormone. The most striking difference between the singing in normal males and stimulated females was in the greater sound volume produced by the males. Whether or not the tone quality was as good as that of the males must be left for experts to decide but certainly the range of tones, variations, trills and duration of song were similar to those of the males of this strain of birds. The temperamental nature of the time and frequency of singing, so characteristic of the males, also occurred in females. The best results were obtained when the females were isolated.

Several canary owners have informed me that female canaries normally will sing under certain conditions. This was not observed in the females used in these experiments nor has it been observed by Mr. Frederichs. The stimulation of the female birds to sing by male hormone injections under the above conditions of the experiments indicates that singing is a secondary sexual characteristic of male canaries.

10632 P

A Method for Concentrating Serum in Cellophane Bags and Simultaneously Removing Salts and Other Constituents.

WILLIAM THALHIMER.

From the Manhattan Convalescent Serum Laboratory and the Bureau of Laboratories, New York City Department of Health.

A report was made about a year ago describing a simple, inexpensive method for concentrating serum under sterile conditions in sterile, cellophane sausage casings.[1] Subsequently, Dr. P. A. Kober[2] informed me that he had described this process in 1917 and 1918 under the name "Pervaporation".

The casing filled with serum is suspended by the tied end from a hook either at room temperature or in a large mechanical refrigerator

1 Thalhimer, William, PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 639.

2 Kober, P. A., *J. Am. Chem. Soc.*, 1917, **39**, 941; 1918, **40**, 1226; PROC. SOC. EXP. BIOL. AND MED., 1917, **15**, 1233.

kept at 5°C, the bag being placed in front of an electric fan. The serum can be concentrated to one-third its volume in about 48 hours at room temperature, and after a somewhat longer interval in a refrigerator. We have also concentrated ascites fluid by this method to as much as one-tenth its original volume. The concentrated serum or ascites fluid is then passed through a Buchner paper pulp filter, and subsequently, a Berkefeld filter. It is then put into sterile vials, and tested for its sterility.

By this method of concentration only moisture and gases are removed; all other substances, including salts, and non-protein nitrogenous materials are concentrated to the same degree as the protein.

It seemed advisable to have available, if possible, a simple method whereby protein constituents could be concentrated with most of the salts and non-protein nitrogenous materials removed, since in conditions such as nephrosis it is manifestly undesirable to inject large amounts of salts or non-protein nitrogenous substances.

The problem of concentrating serum and at the same time dialyzing out most of the salts was solved by immersing the bag of serum in a very pure, inexpensive, colorless commercial corn syrup.* It was found that the serum concentrated more rapidly by this technic than by the air method, and that, at the same time, a great deal of sodium chloride and non-protein nitrogenous materials dialyzed out. Some of the results are given in Table I.

In has been found that only from 5% to 8% of dextrose dialyzes into the bag, and either no dextrin at all or only a trace. The dextrose which dialyzes into the serum might have the advantage of acting as a diuretic.

To determine whether a solution of dextrin could be injected intravenously with safety very small amounts of 50% solution of corn syrup were injected intravenously at first into some patients with hypertension and increased intracranial pressure and later larger amounts. Dr. Francis Murphy of Milwaukee undertook to compare the effect of this solution under these conditions with 50% sucrose solutions. He reports no harmful results or reactions after the intra-

* The corn syrup used is Three Star Corn Syrup, and was generously furnished by the Corn Products Refining Company. The formula given for the corn syrup is as follows:

Moisture		19.5 %
	On Dry Basis	
Reducing Sugars—Dextrose		40 %
	Maltose	2 %
Dextrins		57.75%
Ash		.25%

TABLE I.

Ascites Fluid, Pool II	Unconcentrated %	Concentrated 5.5 times in air %	Concentrated 9.5 times in corn syrup %
Total Protein	2.4	14. (13.2)*	25. (22.8)
Albumin	1.5	8.5 (8.25)	16. (14.25)
Globulin	0.9	5.5 (4.95)	9. (8.55)
Sodium Chloride	—	3.50	1.31 (6.04)
Non-Protein N	0.034	0.144 (0.187)	0.117 (0.323)
Dextrose	—	—	—

Ascites Fluid 3	Unconcentrated %	Concentrated 6.5 times in corn syrup %
Total Protein	3.04	17.9 (19.76)
Sodium Chloride	1.05	0.795 (6.82)

Human Serum No. 206	Unconcentrated %	Concentrated 3 times in air %	Concentrated 3 times in corn syrup %
Total Protein	7.3	22.53 (21.9)	22.77 (21.9)
Sodium Chloride	0.573	1.632 (1.719)	0.771 (1.719)
Non-Protein N	0.032	0.108 (0.096)	0.048 (0.096)

*Figures in parenthesis show the percentage calculated on the basis of concentration. The sodium chloride and non-protein nitrogen actually present in the materials concentrated in corn syrup are much less than the expected amounts because of dialysis out into the corn syrup.

venous injection of large amounts of 50% corn syrup solution.†

Ascites fluid is concentrated just as easily as serum by this technic.

Clinical conditions in which the intravenous injection of serum or ascites fluid concentrated by this technic might be of therapeutic value are nephrosis, surgical shock, conditions of increased intracranial pressure caused by injury, brain tumors, etc.

Reactions have not been caused by the intravenous injection of human serum concentrated by the corn syrup technic.‡

† The 50% corn syrup solution was kindly prepared by Dr. Haldane Gee, of the Sterisol Ampoule Corp.

‡ I am indebted to Sophronia A. Myron and Thelma Schwartz for their valuable technical assistance.

10633 P

A Simple, Inexpensive Method for Drying Serum in the Frozen State in Cellophane Bags.

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A number of methods have been devised for drying serum and other fluid materials while in the frozen state.[1, 2] These methods, though excellent, are somewhat complicated and require moderately expensive apparatus. A simpler, less expensive method would be useful.

Since serum can be concentrated in a cellophane bag by evaporating the moisture in front of an electric fan either at room temperature or in a refrigerator at 5°C, it was decided to find out if the serum would evaporate to dryness when kept continually in a frozen state in the refrigerator. The refrigerator available maintains a temperature of 14°F, that is, 18°F below freezing.

Serum was placed in cellophane bags described in the preceding communication and suspended in the refrigerator. The serum froze rapidly and remained frozen. It eventually became completely dry and this dry residue had the same spongy consistency and light color as serum dried by the lyophile process. It redissolves rapidly, in a minute or two, when distilled water is added, forming a clear or practically clear solution, with the color and appearance of the original serum.

Small sausage casings, about 1 cm in diameter, made out of very thin cellophane allowed about 10 cc of serum, under the above conditions, to evaporate completely to dryness in several weeks. However, it took months for about 100 cc of serum to dry completely in a thicker walled sausage casing whose lumen was about 5 cm.

Some further experiments were carried out keeping the bag of frozen serum under lowered atmospheric pressure and in the presence of a drying agent, such as calcium chloride. By this means, it was possible to reduce considerably the time necessary for drying the serum.*

1 Elser, W. J., Thomas, R. A., and Steffen, G. I., *J. Immun.*, 1935, **28**, 433.

2 Flosdorf, E. W., and Mudd, Stuart, *J. Immun.*, 1935, **29**, 389.

* I am indebted to Sophronia A. Myron and Thelma Schwartz for their valuable technical assistance.

10634

Effect of an Excess of Salt on Resistance to Histamine in Rats.

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In the previous paper[1] it was noted that depletion of the stores of sodium chloride decreased the natural resistance of histamine.

In the present study the prophylactic effect of the administration of an excess of saline to normal rats was observed on the subsequent resistance to histamine. A number of normal adult rats were each given 20 cc of physiological salt solution intraperitoneally. All the saline was absorbed within 3 to 4 hours. The rats were tested 4 hours after injection of the saline with varying quantities of histamine intraperitoneally injected. Control rats were inoculated at the same time. The minimal lethal dose was determined in both groups.

In some instances 2 to 3 cc of fluid was still present in the peritoneal

TABLE I.

Effect of an Excess of Saline on Resistance to Histamine in Rats.* (Combined data of several experiments.)

No. rats	Histamine in mg per kg	Survived	Died
Rats given 20 cc physiological NaCl solution intraperitoneally 4 hr prior to test.†			
2	1200	2	0
4	1400	4	0
2	1600	2	0
2	1800	1	1
5	2000	4	1
3	2200	2	1
2	2400	0	2
Rats fed 2 g salt per day for 3 days prior to test.‡			
3	1400	3	0
8	1600	3	5
7	1800	0	7
4	1900	0	4
Control rats.			
4	1000	2	2
2	1100	0	2
4	1200	0	4

[1] Perla, D., and Sandberg, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 275.

* All the rats in the experiments were of our standard normal Wistar stock, raised in the laboratory under the same conditions of diet for many years. They were free from known latent infections. The histamine was administered intraperitoneally dissolved in small amounts of distilled water.

† In these rats the serum sodium remained at the normal level but the serum potassium rose somewhat. This will be discussed in a subsequent report.

‡ These rats lost about 10 g in weight in 3 days. They drank excessive quantities of water and diuresis was pronounced. The normal diet contains in addition to the traces of Na in the natural foods, one percent by weight of sodium chloride.

cavity 4 hours after injection of the saline. This residual fluid was fairly rich in protein. Blood counts and determination of hemoglobin before and after the injection of the saline showed no evidence of altered concentration of the blood elements. There was no change in the hemoglobin or the total red cell count.

The rats receiving saline withstood 2200 mg of histamine per kilo of body weight, an amount equal to twice that which is lethal for the normal rat (1100 mg per kg). The results are given in Table I.

Feeding salt in amounts of 1 to 2 g per day for a period of 3 days prior to the test also raised the resistance of the rat but not to the same degree as the saline injections. Some rats survived 1600 mg of histamine per kilo of body weight.

Summary. An excess of salt for short periods above the apparent requirement in rats in which the depôts of saline solution have been filled enhances the natural resistance of the animal to large amounts of histamine. The intraperitoneal injection of large quantities of saline a few hours prior to the test increased the resistance to twice the normal. Salt feeding for several days prior to the test raised resistance about 30 to 40%.

10635

Whey as the Substratum in Vitamin B_1 Assays.

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To meet the requirements for the other essential substances of the Vitamin B complex in Vitamin B_1 (thiamine) assays, early workers used autoclaved yeast. Because of the uncertainties involved in obtaining a yeast quite free from the vitamin in question, the use of autoclaved whey and autoclaved liver has been advocated since the thiamine in these apparently is more readily destroyed. For these assays it is customary to place the test animals on the basal ration at weaning, hold them until the body stores of B_1 are depleted, manifested by stationary weight, and subsequently for a given specified period add the material to be tested in amounts sufficient for considerably less than the optimum gain. Although theoretically it should be possible to use a test animal for successive assays, in practice it is customary to make only one assay with a given animal. Under these

conditions, possible discrepancies in the basal ration may be masked, or the results may be modified by enrichments in the test material. Furthermore, when the basal ration is made up on the percentage basis, the amounts taken will vary with the appetite of the animal. Thus, in certain instances, less than the optimum amount of the other constituents of the Vitamin B complex may be taken. Therefore, to make sure that the basal ration is adequate, several successive assays should be made with the same animal, allowing sufficient time between tests for the animal to become depleted of its B_1 store. This would seem to be important, even though a negative control accompany the assays.

To determine the value of certain basal rations for Vitamin B_1 assays, successive tests have been made with animals fed *ad libitum* a basal ration consisting of B_1-free casein 20 g, sucrose 62 g, Crisco 10 g, cod liver oil 2 g, and 4 g Hawk and Oser's salt mixture[1] with iron citrate and copper sulphate. In addition, each animal received daily weighed amounts of those substances: autoclaved yeast, autoclaved liver, and autoclaved whey, used to supply the other components of the Vitamin B complex in these assays. These daily doses were consumed before the basal ration was given. After the weight of the animal had become stationary, Vitamin B_1* was given during a 10-day period. In general, the animals continued to gain for several days thereafter, thus making the assay period conform more nearly to the 14-day period suggested by Coward.[2]

The autoclaved dried whey† was prepared by mixing with distilled water, straining through a fine wire mesh and autoclaving in shallow pans at a depth of ¼ inch for 2 hours at 18 pounds. This was subsequently dried and ground. The yeast, a Northwestern dehydrated product, was mixed in 200 g lots with distilled water, rubbed through a fine mesh strainer, diluted with 300± cc N/10 NaOH to bring to pH 7, and boiled 5 minutes. After standing 24 hours, this was again strained and adjusted to pH 7 with added NaOH. The mixture was then autoclaved 5 hours in ¼ inch layers at 18 pounds, subsequently dried and pulverized. After autoclaving, the pH was between 6.8 and 7. The liver (young beef) was finely ground, mixed with distilled water, autoclaved 20 minutes in ¼ inch layers at 18 pounds, dried at room temperature and ground.

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1 Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

* Betaxin, supplied through the courtesy of the Winthrop Chemical Company, Rensselaer, New York.

2 Coward, K. H., *The Biological Standardisation of the Vitamins*, William Wood and Co., Baltimore, 1938.

† Kindly supplied by the Western Condensing Co., San Francisco, Cal.

TABLE I.
Influence of Various Supplements on Bio-assay of Thiamine.

No. of Animals	Assay period	Supplements: Autoclaved whey	Supplements: Autoclaved yeast	Supplements: Non-autoclaved whey	Avg wt: Initial	Avg wt: End	Avg wt: Gain	Thiamine: Total	Thiamine: For 1 g gain	Thiamine: Range
		g	g	g	g	g	g	γ	γ	γ
4	I		.5	—	77	94	17	39	2.3—	2.1-2.9
3	II		.5	—	88	104	16	39	2.4	2.4-2.7
1	III		.5	—	76	96	20	41	2.1	
3	I	.5	.3	—	80	101	21	48	2.3	1.3-3.5
3	II	.5	.3	—	96	114	18	28	1.6	1.5-2.0
3	III	.5	.3	—	109	125	16	30	1.9	1.4-2.5
1	IV	.5	.3	—	119	134	15	36	2.4	
1	V	.5	.3	—	124	141	17	53	3.1	
3	I	.5	.2	—	77	94	17	43	2.5	1.8-3.2
3	II	.5	.2	—	92	114	22	43	2.0	1.8-2.3
3	III	.5	.2	—	111	128	17	37	2.2	1.9-2.5
1	IV	.5	.2	—	116	136	20	54	2.7	
2	I			.5	66	92	26	169	6.4	6.3-6.4
4	I	.5		—	58	72	14	80	5.7	4.6-7.7*
4	II	.5		—	70	82	12	224	18.6	10.4-28.0
4	III	.5		—	81	89	8	421	52.6	50.0-123.5
4	IV	.5		—	120	135	15	152	10.1	7.0-18.3†
4	V	.5		—	133	141	8	499	62.4	45.0-110.5
5	I	.8 Autoclaved liver		—	67	74	7	155	22.1	15.0-26.3
2	I	.5		—	82	102	20	47	2.4	2.3-2.4

*Animals were given 0.1 g yeast for each of 6 days previous to test to stimulate appetite. No gain during period.
†During 20 days of depletion, animals were given 8 daily doses of 0.5 g autoclaved yeast.

The thiamine which was dissolved in 0.2% HCl solution which previously had been boiled and cooled was stored in a low temperature refrigerator (8°C). To prevent contamination, the pipette used for measuring was kept when not in use in 95% alcohol. The doses of thiamine, which were given first in the day's program to insure complete consumption, were mixed with a small amount of distilled water and a few grains of sugar.

Results. Successive assays of thiamine with the basal ration and 0.5 g autoclaved yeast are consistent from period to period, averaging 2.3γ for one gram of gain; successive assays with the basal ration and 0.5 g autoclaved whey required an increasingly large amount of thiamine for one gram of gain (Table I). That the increase during the succeeding assays in the thiamine requirement is not related to depleted stores of Vitamin B_1 but to a decrease in some substance necessary for growth which is in low concentration in whey is indicated by the animals receiving 0.5 g autoclaved whey and, in addition, 0.2 g and 0.3 g respectively of autoclaved yeast. The amount of B_1 required for one gram of gain is comparable to that required with 0.5 g autoclaved yeast. The inadequacy of whey is further shown by the animal which was placed at weaning on the basal ration with 0.8 g autoclaved whey daily and in addition 2 cc (20γ) thiamine. During the first 10-day period, the animal gained 12 g; during the second and third 10-day periods, he gained 7.4 and 1.0 g respectively. The omission of the thiamine at the end of the third period and the addition of 0.2 g autoclaved yeast daily resulted in a gain of 11.7 g during the subsequent 10-day period (Table II).

The performance of the 4 animals (Table I) which were subjected to 5 successive assays of B_1 with depletion periods between, again shows the inadequacy of whey. Six days previous to Assay I, all animals which had been holding on the basal ration with 0.5 g autoclaved whey were given 0.1 g dried yeast daily to stimulate their appetites; during this period there was no gain in weight. The Vitamin B_1 requirement for one gram of gain was 5.7γ for the first period and 18.6γ and 52.6γ for the 2 subsequent periods. Previous to Assay IV, the animals were given during 20 days of depletion 8 daily doses of 0.5 g autoclaved yeast. Following this, the vitamin required for one gram of gain decreased. During the subsequent period when only autoclaved whey was given during the depletion period, the thiamine requirement seemingly for one gram of gain increased to a value more nearly comparable to the third assay period. Apparently the animals failed to store enough of the unknown factor present in yeast to carry over to the subsequent assay period. In all

TABLE II.
Successive Assays of Thiamine with Autoclaved Whey—No Depletion Periods.

No. of Animals	Assay period	Supplements		Avg wt			Thiamine	
		Autoclaved whey	Autoclaved yeast	Initial	End	Gain	Total	For 1 g gain
		g	g	g	g	g	γ	γ
1	I	.8	—	79.5	91.5	12.0	210	17.5
1	II	.8	—	91.5	98.9	7.4	240	32.4
1	III	.8	—	98.9	99.9	1.0	320	320.0
1	IV	.8	.2	98.9	110.6	11.7	—	—

probability, no more thiamine is needed for gain under one condition than under another, but in an attempt to produce gain in animals depleted of their stores of the unknown factor, more thiamine was used.

Tests during first assays with autoclaved liver were comparable to those with autoclaved yeast. This unknown essential complex is in high concentration in both yeast and liver. It is not destroyed by autoclaving at pH 7 under 18 pounds pressure. Whey contains little, 0.8 g having only slightly more than 0.5 g.

The nature of the substance or substances which are in low concentration in whey has not been investigated. The retarded growth with no other obvious symptoms in animals fed the basal whey ration with repeated doses of Vitamin B_1 during a 3-month test period suggests that one component of Factor W (Frost and Elvehjem),[3] which seems identical with the filtrate factor of Edgar and Macrae,[4] and Edgar, El Sadr, and Macrae,[5] may be the substance in question. In line with this are the findings of Jukes and Richardson[6] to the effect that dried whey is not a concentrated source of the filtrate factor.

Although we have presented no data to this end other than growth and lack of growth, during the course of our experiments we have noted that the animals which received the basal whey ration lacking either B_1 or the unknown factor or both, ate little. It was only when the two were included in the ration that there was any appreciable food consumption. Seemingly appetite hitherto related to B_1 is concerned not alone with B_1 but with a combination of B_1 and this unidentified factor.

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[3] Frost, D. L., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **121**, 255.

[4] Edgar, C. E., and Macrae, T. F., *Biochem. J.*, 1937, **31**, 886.

[5] Edgar, C. E., El Sadr, M. M., and Macrae, T. F., *Biochem. J.*, 1938, **32**, 2200.

[6] Jukes, T. H., and Richardson, C. A., *J. Agr. Res.*, 1938, **57**, 603.

10636

Identification of a Strain of Poliomyelitic Virus from Feces in Non-Paralytic Poliomyelitis. I. Immunologic Tests.*

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In 1937 we recovered a virus from the stools of a child with non-paralytic poliomyelitis.[1] Stools passed on the 1st, 14th, and 25th days after the onset of his mild illness were positive, but virus was not recovered from 2 specimens passed on later dates. For the original identification of this virus, 3 criteria were used: (i) The production of the usual signs of experimental poliomyelitis in monkeys; (ii) the demonstration of typical histological lesions in the monkey's spinal cord; (iii) passage to another monkey. Subsequently this strain, known as the SK strain, has been passed to its 8th generation and has produced typical experimental poliomyelitis in more than 40 monkeys. Intracerebral, intraperitoneal, intracutaneous, intranasal and oral inoculations have been successful.[2]

Further identification of this virus is presented in this report which includes the results of neutralization tests, reinoculation tests in monkeys, and also susceptibility tests in animals usually resistant to poliomyelitis.

The neutralization tests appear in Table I. "Anti-sera" prepared by immunization of monkeys with 6 different strains of poliomyelitic virus, whose properties have been described previously,[3] were used. Two series of tests were run: one on April 21, 1938, with the strain in its first generation; the other on November 8, 1938, with the strain in its fourth generation and with its infectivity considerably enhanced. In all neutralization tests equal parts of a suspension of 10% virus, and undiluted serum were mixed and incubated for 2 hours at 37°; 0.5 cc of the resulting mixtures was then inoculated intracerebrally into one monkey each. With each series there was

* Aided by grants from the President's Birthday Ball Commission for Infantile Paralysis Research and from the National Foundation for Infantile Paralysis.

1 Trask, J. D., Vignec, A. J., and Paul, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 147; *Ibid., J. Am. Med. Assn.*, 1938, **111**, 6.

2 Vignec, A. J., Paul, J. R., and Trask, J. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 246.

3 Trask, J. D., Paul, J. R., Beebe, A. R., and German, W. J., *J. Exp. Med.*, 1937, **65**, 687.

TABLE I.

Neutralization Tests Between "Antisera" for 6 Strains and SK. Virus in Its 1st and 4th Generations.

*Immune sera prepared from following strains	1st Test—(April 21, 1938) SK. virus: Generation 1 (pool of cords of No. 849, No. 860, and No. 901)					2nd Test—(Nov. 8, 1938) SK. virus: Generation 4 (Cord of No. 1020—*M. mordax*)				
	Dose 0.5 cc, %	No. *Mac. rhesus*	Result	First day of Fever	First day of Paral.	Dose 0.5 cc, %	No. *Mac. rhesus*	Result	First day of Fever	First day of Paral.
Aycock, 1920	5	960	—			5	1076	—		
Park, Mixed	5	961	P	12	14	5	1078	P	7	13
Flexner, 1931	5	962	—			5	1082	P	7	10
We., 1931	5	964	P	15	18	5	1083	P	5	9
Wfd., 1934	5	966	—			5	1084	—		
McC., A., 1934	5	967	—							
McC., B., 1934						5	1085	—		
Control Sera:										
Convalescent Human	5	939	—			5	1074	—		
Normal Monkey	5	948	P	7	12	5	1086	P	5	9
,, ,,	5	968	—			5	1087	P	5	9
,, ,,	5	971	P	12	14					
,, ,,	0.5	972	—			0.5	1088	P	8	12
,, ,,	0.5	973	—			0.5	1089	P	7	14

Legends.—Result: P = The monkey contracted experimental poliomyelitis and therefore the virus was not neutralized.

— = The monkey did not contract experimental poliomyelitis and therefore the virus was neutralized, or (as in the case of titrations) it was of insufficient virulence (or dosage) to infect.

First Day of Fever: 12 = Fever began on the 12th day of the experiment.

*For their adequate interpretation these results should be compared with our previous cross immunization tests.[3] It may suffice to say that in such tests, 5 sera neutralized their homologous strains. The We. and McC. (B) samples have not been described before. However, the former neutralized the Park and Flexner strains in June and July, 1936, and was not tested with its own strain.

included: (i) a control neutralization test with human serum (made up of pooled samples from 5 convalescent paralytics from the epidemic of 1931), and (ii) infectivity controls in which virus suspension of 2 different concentrations were mixed with normal monkey serum. In the second series of tests (November, 1938) the infectivity of the virus proved to be at least 10 times greater than that used in the first. What differences exist between the results of the 2 series seem largely explainable on this basis.

The results in Table I indicate that the SK. strain is related immunologically to the Aycock strain of 1920 and to the Wfd. and McC. strains which came from the California epidemic of 1934. On the other hand, there was at least some immunologic difference between the SK. strain and the Park strain and two eastern strains of 1931.

The series of reinoculation tests, in which the SK. strain was tested on monkeys which had been paralyzed previously by various poliomyelitis viruses, appear in Table II. Here, again, 2 sets of tests were run. In the first, with the weaker (1st generation) material of April, both the paralyzed, heterologously "immunized" monkeys, as well as the homologously immunized monkeys, survived reinoculation with the SK. strain without infection. However, not all of the normal controls were infected in this series. In the second series with the stronger (4th generation) material of November, 4 of the 6 para-

TABLE II.
Reinoculation of Paralyzed Monkeys Convalescent from Homologous and Heterologous Strain Infections.

Convalescent Monkeys				Reinoculation with SK. strain 0.5 cc. 5% virus intracerebrally	
Date of first paralysis, 1938	*M. rhesus* No.	Strains (All 1937)	Source of Strain	April 21, 1938* Paralysis	Nov. 8, 1938* Paralysis
2/20	921	SK. New Haven Co.	Feces	—	—
3/31	940	G.W. " " "	Spinal cord	—	—
4/13	934	Wn. " " "	" "	—	—
4/22	954	G.W. " " "	" "	Not done	—
7/27	996	Toomey, Cleveland†	" "	" "	+ (mild, delayed 3 days)
9/1	995	Ah., Toronto†	" "	" "	+ (mild, delayed 5 days)
9/11	973	Fx., Toronto†	" "	" "	—

*Same lots of virus as those used in experiments in Table 1. (See virulence controls.)

†We are indebted to Dr. L. N. Silverthorne of the Hospital for Sick Children, Toronto, and to Dr. J. A. Toomey of the City Hospital, Cleveland, for 3 strains of virus.

TABLE III.
Inoculation of Rabbits, Guinea Pigs, and Swiss Mice.

Date, 1938	Animals	Total dose of SK. virus* cc	%	Routes	Results Symptoms during 4 wks	Histology Brain or Cord	Meninges
Apr. 21	2 rabbits	1	5	Intracerebral	1 remained well	—	—
				Intraäbdominal	1 wry neck	+	+
				Corneal			
Apr. 21	4 guinea pigs	0.7	5	Intracerebral	4 dead with peritonitis on 3rd, 6th, 10th and 25th days	All negative	
				Intraäbdominal			
				Corneal			
Apr. 21	6 mice	0.03	5	Intracerebral	Remained well	,,	,,
June 1	2 rabbits	0.5	10	,,	1 remained well	—	—
					1 dead—brain abscess, 7 days	—	Purulent
June 1	3 guinea pigs	0.2	10	,,	1 remained well	—	—
					1 brain abscess	—	Purulent
					1 equivocal fever	—	—
Nov. 8	2 rabbits	0.35	10	,,	1 remained well	—	—
				Corneal	1 snuffles	—	—
Nov. 8	3 guinea pigs	0.1	10	Intracerebral	3 equivocal fever: onset 11th and 12th days; 1 sick with pelvic abscess, killed 28th day	—	—
						—	—
						—	—
Nov. 8	6 mice	0.03	10	,,	5 remained well	5 negative	
					1 dead 3d day	+	+
Dec. 15	12 mice	0.03	10	,,	Remained well	All negative	

*For control of activity of virus used April 21st and November 8th, see Table I; virus used June 1st was active in a monkey inoculated that day, but a larger dose was used; 4 cc—10% virus; virus used December 15th was freshly prepared from the cord used November 8th, see Table I.

+ = Accumulation of round cells. — = No lesions.

lyzed, heterologously "immunized" monkeys survived reinoculation without infection. Two, however, out of these 6 "immune" monkeys (Nos. 996 and 995) developed mild, delayed attacks of poliomyelitis. The heterologous reinfection of monkeys with poliomyelitic virus has been described.[4, 3] This indicates an immunologic similarity among strains from New Haven County in the autumn of 1937. It appears to suggest a difference between the SK. strain and strains collected during the outbreaks in Toronto and Cleveland in 1937, though obviously factors other than immunologic differences might have determined the results. Immunologic similarity among strains from the same epidemic have been described before by means of neutralization tests.[3]

As a third means of identification (or of eliminating other viruses) rabbits, guinea pigs and Swiss mice were inoculated. These tests appear in Table III. After recovery from the trauma of inoculation, these animals presented no consistent or characteristic signs or symptoms, and no consistent microscopic evidences of diffuse or localized nonsuppurative lesions. A variety of lesions were detected in some of the 40 animals which were used in these experiments. Some of these were thought to result from the trauma of inoculation. Some were unexplained. None of them have been attributed to the SK. virus and we have presumed that this virus was non-infective for these 3 species by the routes and in the doses used. No reactions were produced in the eyes of 4 rabbits inoculated on the scarified cornea.

Summary Strains of virus, recovered from the stools of a child (SK.) with nonparalytic poliomyelitis, produced typical experimental poliomyelitis in *Mac. rhesus* monkeys. This SK. strain appears to be related immunologically to other strains of poliomyelitis virus recovered from the same epidemic, and to be related to the Aycock strain of 1920, and to some Californian strains of 1934. On the other hand, there appears to be at least some immunologic difference between the SK. strain and the Park strain, two eastern strains of 1931, and strains from Toronto and Cleveland of 1937.

The SK. strain produced no corneal reaction in rabbits and no consistent type of infection on intracerebral inoculation into rabbits, guinea pigs, and Swiss mice.

These tests yield further evidence, in addition to that already reported,[1] that the SK. strain is an example of poliomyelitic virus.

4 Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1933, **58**, 513.

10637

Poliomyelitic Virus from Feces in Non-Paralytic Poliomyelitis. II. Infectivity by Various Routes.*

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In the preceding paper,[1] immunologic reactions and the non-infectivity for small laboratory animals of a strain of virus from human feces, known as the SK. strain of poliomyelitic virus were described. The results of inoculation of several species of monkeys by a variety of routes with this SK. strain is the subject of this note.

An abbreviated chart of the genealogy of the SK. strain appears in Fig. 1. This shows the source and generation of the strain used in these multiple route experiments. The positive results appear in more detail in Table I. In using this method as a means of describing the properties of a given strain, it is recognized that different strains of poliomyelitic virus and even different generations of the same strain vary somewhat in their ability to infect by multiple routes.[2] Cutaneous infectivity, for instance, has been noted in some strains recently isolated from a human source, whereas this property has not been prominent in several others.[3]

In its fourth generation, and at the time of the first multiple route experiment, the SK. strain was of moderately high intracerebral virulence (*cf.* virulence test in Nov. neutralization experiment in preceding paper[1]). It proved, at this time, to be unusually infective by the intracutaneous, intratonsillar,[4] and intranasal routes in that the incubation periods in monkeys inoculated by these routes were the same as by the intracerebral route, namely, 7 days (Table I). Oral infections occurred in the 5th and 6th generations. Later the strain did not infect by routes other than the intracerebral, Fig. 1.

As part of the multiple route experiment, 2 examples of inducing the experimental disease by feeding are presented. Experiments of

* Aided by grants from the President's Birthday Ball Commission for Infantile Paralysis Research and from the National Foundation for Infantile Paralysis.

1 Trask, J. D., Paul, J. R., and Vignec, A. J., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 241.

2 Trask, J. D., Paul, J. R., German, W. J., and Beebe, A. F., *Trans. Assn. Am. Phys.*, 1937, **52**, 306.

3 Trask, J. D., and Paul, J. R., *Science*, 1938, **87**, 44.

4 Sabin, A. B., *J. Am. Med. Assn.*, 1938, **111**, 605.

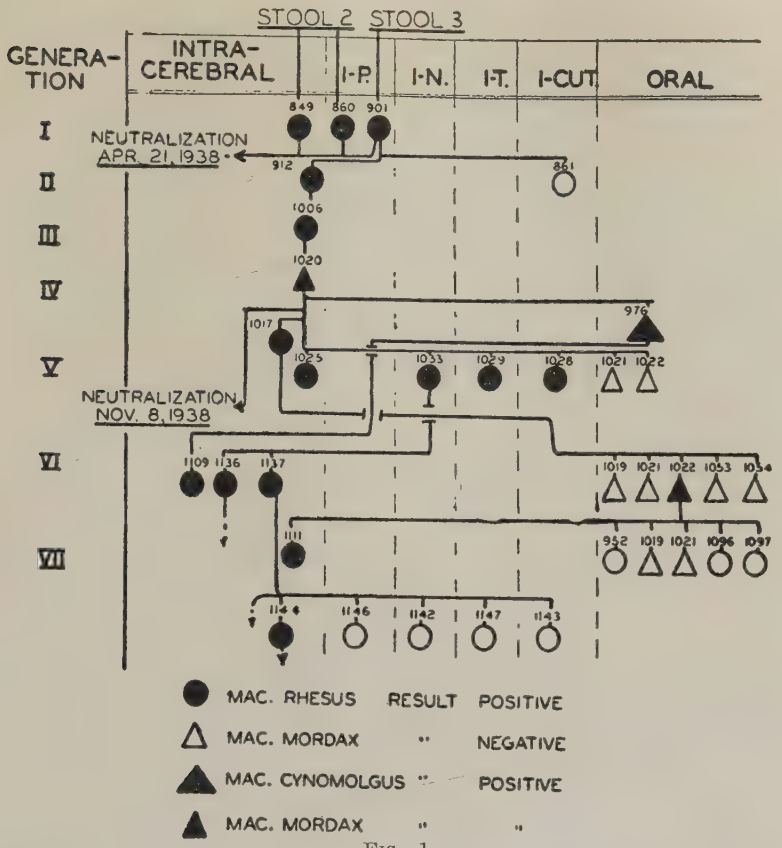

FIG. 1.

Diagram of various passages of the SK. strain with results of its administration by various routes. These are indicated by headings at the top of the chart: I-P = Intraperitoneal; I-N = Intranasal; I-T = Intratonsillar, and I-cut. = Intracutaneous.

All positive results indicate a disease typical of experimental poliomyelitis with characteristic histological findings at autopsy. They are represented by black symbols while negative results appear as open symbols.

this type with poliomyelitic virus have, in general, been unsuccessful in this country.[5] The only previously reported positive result is that of Saddington, who reproduced the disease in a *Mac. cynomolgus* monkey by feeding large amounts of virus in milk (30 cc of a 10% suspension on 6 successive days).[6] European authors, on the other hand, have recorded a number of positive results by this route, and Kling and his associates have emphasized the importance of using the

5 Clark, P. F., Roberts, D. J., and Preston, W. S., Jr., *J. Prev. Med.*, 1932, **6**, 47. Also, Lennette, E. H., and Hudson, N. P. *J. Infect. Dis.*, 1936, **58**, 10; and Flexner, S., *J. Exp. Med.*, 1936, **63**, 209.

6 Saddington, R. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 838.

TABLE I.
Positive Result Experiments.

Date, 1938	Route of inoculation	Total dose	Monkey No.	Species	Result			
					Incubation period—days*	Paralysis	Lesions	Passage
Sept. 9	Oral	3 g†	976	*Mac. cynom.*	11	++++	++	+
Oct. 21	"	0.9 g‡	1022	*Mac. mordax*	19	++++	+	+
Sept. 21	Intracerebral	0.5 cc of 10%	1025§	*Mac. rhesus*	7	+++	+	
" 21	Intracutaneous	2 " " "	1028	"	7	+++	++	
" 21	Intranasal‖	4 " " "	1033	"	7	++	+	+
" 21	Intratonsillar	2 " " "	1029	"	7	+++	±	

*Computed from the first day of feeding or inoculation and counting this day as 1.
†Equivalent to 30 cc of a 10% suspension.
‡ " " 9 " " " " " "
§No. 1025 was sick with tuberculosis.
‖Instilled into nares on 2 successive days.

Mac. cynomolgus for this purpose.[7] Reference to Fig. I will reveal that this species and the *Mac. mordax* were included in our experiments.† [8] Since finding that the strain would infect these species by feeding, we have fed at varying intervals, but without success, a total of 8 *Mac. rhesus* monkeys (3 of which appear in Fig. 1).

In conducting the feeding experiment, fillets of infected spinal cord were inserted into bananas. These were given in 3 successive days, no other food being provided until after the virus-contaminated banana had been eaten. A brief clinical-pathological description of the 2 successful feeding experiments follows:

1. Monkey No. 976, *Mac. cynomolgus,* received 1 g of infected cord on Sept. 9, 10, and 11, 1938, respectively. On Sept. 19th he developed fever, a coarse tremor, and was unusually quiet. It was also noted at this time that his voice seemed weak. The disease progressed rapidly and within the next 24 hours his neck, back, and right arm were definitely weak; he had a right facial paralysis and his voice was entirely gone. The following day the animal was prostrate and was sacrificed.

At autopsy, no evidence of gross pathology was noted. A careful search was made throughout the intestinal tract for possible erosions or abrasions. The mesenteric glands were pale and not enlarged. Histologically the medulla, cervical, thoracic, and lumbar cord showed typical and extensive lesions of experimental poliomyelitis. At the periphery of the olfactory bulbs, large, foamy cells were surrounded by an inflammatory reaction. This reaction consisted of necrotic cells, a few polymorphonuclears, and many small round cells. Passage to a second monkey by means of intracerebral inoculation of a 10% suspension of the spinal cord was successful.

2. Monkey No. 1022, *Mac. mordax,* received 0.3 g of virus-infected cord on Oct. 21, 22, and 23, 1938, respectively. On the 19th day he developed fever and appeared nervous. The following day he was tremulous, ataxic, and his voice was weak. The disease progressed slowly in this animal, but by the sixth day of illness, both legs, one arm, and his back were paralyzed; he was sacrificed on this day. Grossly, no lesions were observed. Characteristic histological lesions were found in the cervical and lumbar cord. The olfactory bulbs were negative, but serial sections were not done. Passage to a second

7 Kling, C., Levaditi, C., and Hornus, G., *Bull. Acad. Méd.,* 1934, **111**, 709.

† *Mac. mordax* resembles *Mac. cynomolgus* in appearance but is somewhat smaller. Our attempts to classify the two species exactly have not been successful, but they are apparently very closely related.

8 Elliott, D. G., *A Review of the Primates,* 1913, Vol. 2, 185.

monkey by means of the intracerebral inoculation of a 10% suspension of the spinal cord was successful.

These feeding experiments, considered by themselves, serve chiefly to confirm the work of Kling and his associates[7] who have long maintained that poliomyelitic virus will infect the *Mac. cynomolgus* by the oral route, but not the *Mac. rhesus*. In conjunction with the inoculations by other routes, they also indicate that the behavior of this virus is compatible with that of poliomyelitic virus, and these results thus become of supplementary value in the identification of this strain.

Summary. The SK. strain of poliomyelitic virus has been shown to be occasionally infectious by the intraperitoneal, intracutaneous, intratonsillar, and oral routes. The *Mac. mordax* species of monkey, as well as the *Mac. cynomolgus,* is susceptible to infection with poliomyelitic virus by the oral route.

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Detection of Free Polysaccharide in Blood of Pneumococcic Pneumonia Patients; Prognosis and Therapy.

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Early observations by Dochez and Avery[1, 2] and by Blake[3] demonstrated the presence of specific soluble pneumococcal substance in the blood and urine of patients suffering from lobar pneumonia. The substance, a product of the growth of pneumococci, rather than of their degeneration, was identified as capsular polysaccharide by the work of Heidelberger and Avery.[4] Of a total of 44 cases studied by Dochez

* Research Fellow, Pneumonia Service, Harlem Hospital. These studies received financial support from the Metropolitan Life Insurance Company, and from Mr. Bernard H. Baruch, Mr. Bernard M. Baruch, Jr., Miss Belle N. Baruch, and Mr. H. Robert Samstag.

1 Dochez, A. R., and Avery, O. T., PROC. SOC. EXP. BIOL. AND MED., 1917, **14**, 126.

2 Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, **26**, 477.

3 Blake, F. G., *Arch. Int. Med.*, 1918, **21**, 779.

4 Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1923, **38**, 73.

and Avery[2] (25) and by Blake[3] (19), soluble substance was detected in the blood in 11. Ten of these patients died, the 11th having shown only traces in the blood. Blake[3] demonstrated a reciprocal relation between the amount of polysaccharide in the urine and blood, and the development of antibody. It was felt advisable to repeat these observations, particularly noting the comparative influence of sulfapyridine, specific serum, or the combination on the antigen-antibody balance, and its relation to the outcome of therapy. Conceivably, sulfapyridine might decrease the formation of polysaccharide by inhibiting bacterial growth, thus increasing availability of spontaneously produced or passively introduced antibody.

Studies were carried out in the following manner: Repeated blood cultures were taken, using 3-5 cc quantities. For antigen determination, 0.5 cc quantities of serial dilution of patient's serum (using sterile 0.85% saline as diluent) were mixed in 10 x 75 mm tubes with 0.5 cc of a 1 to 10 dilution of homologous rabbit antipneumococcal serum,† having an original antibody-titer of 5,000 to 10,000 units per cc.‡ For agglutination 0.5 cc of saline suspensions of the sediment from 18-hour broth cultures, giving an evident specific Neufeld reaction, were employed. For precipitin-detection, 0.5 cc of freshly-prepared appropriate saline dilutions§ from 1 to 1,000 or 1 to 5,000 stock solutions of homologous polysaccharide‖ were employed, and mixed with 0.5 cc of serially diluted patient's serum. Controls with heterologous polysaccharide and heterologous rabbit antipneumococcal serum; positive and negative controls showing specific and adequate activity of the polysaccharide and antiserum; and saline controls of suspensions of organisms were employed with each determination.

The antigen-antibody balance was determined repeatedly, an average of 5 times each, in 25 cases.¶ No serum polysaccharide was found in 21, all of whom recovered under therapy with sulfapyridine, serum, or both. Capsular polysaccharide was detected in the serum

† Sera used were commercial rabbit sera obtained from several manufacturers; the titers used were those determined by the manufacturers' mouse protection tests.

‡ Recent observations on the use of constant 0.5 cc portions of patient's serum with varying dilutions of added type-specific serum indicate that it is possible to detect capsular polysaccharide in lower concentrations by this procedure than by the method of Blake and of Dochez and Avery.

§ Appropriate dilutions must be determined for each type used, and for each batch of antigen. For types III and VII, 1 to 50,000 dilution was employed throughout.

‖ Supplied through the courtesy of Dr. W. G. Malcolm of Lederle Laboratories, Inc., Pearl River, New York.

¶ Detailed description of these cases will be published.

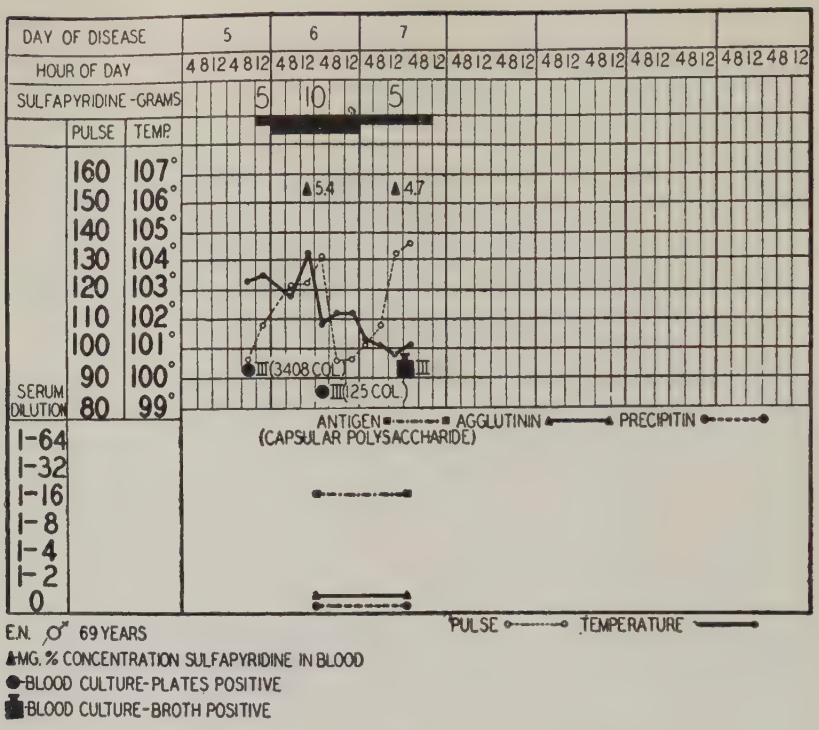
DAY OF DISEASE
5
6
7
HOUR OF DAY
4 8 12 4 8 12 4 8 12 4 8 12 4 8 12 4 8 12 4 8 12 4 8 12 4 8 12 4 8 12 4 8 12 4 8 12
SULFAPYRIDINE - GRAMS
5
10
5
PULSE
TEMP.
160 107°
150 106°
140 105°
130 104°
120 103°
110 102°
100 101°
90 100°
80 99°
SERUM DILUTION
1-64
1-32
1-16
1-8
1-4
1-2
0
▲5.4
▲4.7
●III(3408 COL.)
●III(25 COL.)
ANTIGEN
AGGLUTININ
PRECIPITIN
(CAPSULAR POLYSACCHARIDE)
PULSE
TEMPERATURE
E.N. ♂ 69 YEARS
▲MG. % CONCENTRATION SULFAPYRIDINE IN BLOOD
●BLOOD CULTURE-PLATES POSITIVE
■BLOOD CULTURE-BROTH POSITIVE

FIG. 1.

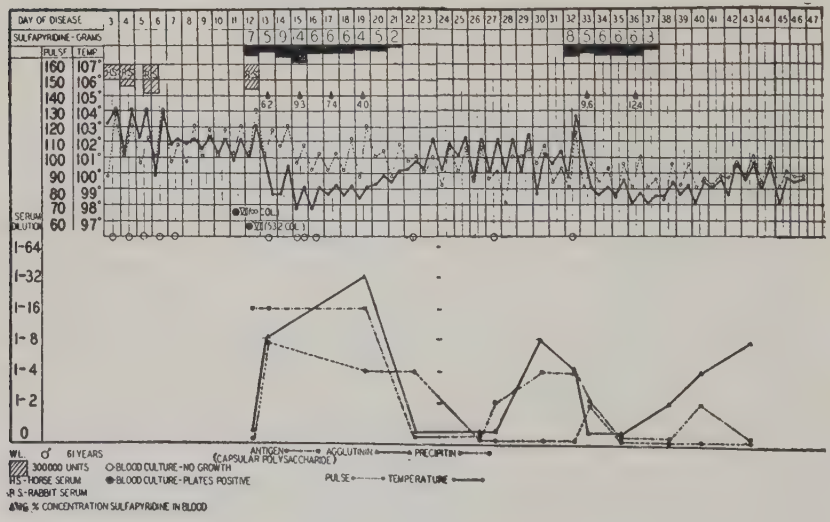
DAY OF DISEASE
SULFAPYRIDINE - GRAMS
7 5 9 4 6 6 6 4 5 2
8 5 6 6 6 3
PULSE
TEMP.
SERUM DILUTION
1-64
1-32
1-16
1-8
1-4
1-2
0
ANTIGEN
AGGLUTININ
PRECIPITIN
(CAPSULAR POLYSACCHARIDE)
PULSE
TEMPERATURE
300000 UNITS
HS-HORSE SERUM
RS-RABBIT SERUM
BLOOD CULTURE-NO GROWTH
BLOOD CULTURE-PLATES POSITIVE
▲MG. % CONCENTRATION SULFAPYRIDINE IN BLOOD

FIG. 2.

of the remaining 4. Three of these were Type III cases, 2 bacteremic; all 3 died. One Type III patient (Fig. 1), a 69-year-old Negro male with bacteremia, died despite 48 hours of therapy with sulfapyridine alone, with no appreciable effect on the circulating polysaccharide and no development of antibody, although the colony-count from the blood culture was progressively reduced. In this case, the supernatant fluid from one of the tubes in which the serum polysaccharide had been precipitated gave evidence, when retested, of complete absorption of its type-specific antibody. The fourth case (Fig. 2) was a 61-year-old Negro male admitted on the 3rd day of a single-lobe Type VII pneumonia, non-bacteremic on admission. Studies were begun after failure to respond to more than 1,000,000 units of Type VII antiserum, both horse and rabbit. At this time the patient had an overwhelming invasion of the blood stream, and a considerable quantity of capsular polysaccharide in the serum, without evidence of antibody. Infection was controlled, and the blood promptly sterilized with sulfapyridine and additional rabbit serum, although polysaccharide persisted in the blood stream, together with detectable precipitin, for at least 8 days. Following cessation of sulfapyridine there was gradual depletion of serum-antibody and a return of detectable polysaccharide, coincident with recurrence of fever, culminating in chill, although demonstrable blood-stream invasion did not recur. However, at this time agglutinins were detectable in the blood. A second administration of sulfapyridine, at a time when both agglutinin and polysaccharide were detectable in the blood, resulted in prompt control of the infection with disappearance of polysaccharide. Cessation of drug therapy the second time was followed by the transient reappearance of polysaccharide and a slight elevation of temperature, together with increasing antibody-concentration and ultimate return of temperature to normal. The patient, though now apparently over the acute infection, has bronchoscopic evidence of a collection of granulation tissue partially obstructing the right upper-lobe bronchus with roentgenographic evidence of atelectasis, and is still under observation.

Of interest is the simultaneous occurrence of pneumococcal polysaccharide and its homologous precipitin in the blood of this patient as well as its prolonged duration. This occurrence in immune rabbits injected intravenously with homologous polysaccharide has not been observed by Downie.[5] Similar occurrences have been observed *in vitro* by Heidelberger and Kendall[6] (pneu-

[5] Downie, A. W., *J. Path. and Bact.*, 1937, **45**, 149.

[6] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1929, **50**, 809.

mococcus III polysaccharide and purified homologous precipitins), and by Taylor, Adair, and Adair[7] (serum albumin and its precipitin). The former authors explained the phenomenon on the basis of the laws of mass action which they derived for the precipitin reaction. Marrack[8] reviews the observations on this occurrence and explains it on the probable existence of non-uniformity of antigen and of antibody.

Conclusions. The occurrence of free polysaccharide in the blood of pneumonic patients is not uncommon. Its presence is generally indicative of a severe infection, with usually fatal outcome. It would appear that sulfapyridine alone may be ineffective in the control of those pneumococcic infections which are accompanied by the production of sufficient polysaccharide to reach relatively high concentrations in the blood stream, but that it may be effective in such instances if it is combined with the administration of sufficient type-specific antibody. The observations reported suggest that sulfapyridine alone may control pneumococcic infections provided there is not too much antigen present in the blood, and some specific antibody is produced.

10639 P

Group Similarity of Alpha Hemolytic Bovine Mastitis Streptococci for Lancefield's Serological Group C.

RALPH B. LITTLE. (Introduced by C. TenBroeck.)

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Minett and his associates[1] included in mastitis streptococcus Group II certain streptococci which usually cause a more acute form of mastitis with less tendency to a permanent infection than Group I. In the serological study of these streptococci, Stableforth[2] found that, although grouped together on the basis of cultural and biochemical similarities, they could also be regarded as a serological group because they were directly or indirectly connected antigenically. Diern-

[7] Taylor, G. L., Adair, G. S., and Adair, M. E., *J. Hyg. Camb.*, 1932, **32**, 340.

[8] Marrack, J. R., *Medical Research Council, Special Report Series*, No. 230, 1938, pp. 146-7.

[1] Minett, F. C., Stableforth, A. W., and Edwards, S. J., *J. Comp. Path. and Ther.*, 1929, **42**, 213.

[2] Stableforth, A. W., *J. Comp. Path. and Ther.*, 1932, **45**, 185.

hofer[3] described the cultural characters of similar streptococci isolated by him, which he designated as *Str. dysgalactiæ*.

Lancefield[4] reported that certain nonhemolytic strains of streptococci were members of the serological Group C. In a personal communication she stated that these nonhemolytic strains were from cases of bovine mastitis.

In the serological study of 15 strains of *alpha* hemolytic mastitis streptococci of Group II *(Str. dysgalactiæ)*, isolated here or obtained from 3 other laboratories, it was found that all 15 strains possessed an antigen which gives a group-specific precipitin-reaction with sera of Lancefield's serological Group C. Furthermore, the group-specific antibodies in Group C sera obtained from Dr. Lancefield could be removed by absorbing with the Group II mastitis strains. Antisera prepared from formalin-killed cultures of Group II streptococci were precipitated by the extracts of hemolytic strains of Group C.

In a personal communication of April 18, 1939, Dr. Wayne Plastridge informed the writer that mastitis strains originally classified by him as *S. pseudo-agalactiæ* belong to serological Group C and are culturally identical with cultures described by Diernhofer[3] as *Str. dysgalactiæ*.

The results show that strains of *alpha* hemolytic mastitis streptococci (Group II) either are related to or belong to Lancefield's serological Group C.

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Effect of Neoprontosil on Bacterial Toxins.

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Domagk[1] first reported that Prontosil prevented the death of mice injected with lethal doses of hemolytic streptococci, even though it had no bactericidal effect on the microörganisms *in vitro*. Because of Domagk's findings, the drug was used for treatment in streptococcal infections in man and subsequently in other infectious diseases.

3 Diernhofer, K., *Milchwirtsch. Forsch.*, 1932, **13**, 368.

4 Lancefield, R. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 473.

1 Domagk, Gerhard, *Deutsche Med. Wchnschr.*, 1935, **61**, 250.

The favorable results obtained in the clinical use of the compound prompted several investigators to study its mode of action. Although sulfanilamide soon became more widely used than Prontosil, the fact that the latter is much less toxic warrants further investigation of its therapeutic value.

Because Neoprontosil* has little, if any, bactericidal effect *in vitro,* our studies have been concerned with the action of the drug on bacterial toxins. This report describes the effect of the compound in mice when it is given orally, intraäbdominally, or subcutaneously after the injection of fatal doses of the following toxins: staphylococcal, streptococcal, gonococcal, and those formed by *Clostridium botulinum* and *Clostridium welchii.* Rockland mice, weighing 20 g each, were used in all the experiments. A 5% solution of Neoprontosil in distilled water was used throughout. The preparation was introduced directly into the stomach by means of a hypodermic syringe equipped with a blunted 18-gauge needle, 32 mm in length. This procedure is referred to hereafter as "oral administration."

Staphylococcal Toxin. The toxins from 3 hemolytic strains of *Staphylococcus aureus* were employed. Two of the strains were recovered from purulent discharges—one from otitis media, the other from a furuncle. The third strain was isolated from the nasal mucosa of a patient with small boils in and about the nose. The toxin was prepared according to Dolman's technic.[2] A 36-hour culture grown on semi-solid agar was mixed with Douglas' broth, filtered through cheese-cloth and filter-paper, and then centrifugalized. The supernate constituted the toxin. Merthiolate was added to give a final concentration of 1:8,000.

The toxin administered orally produced no ill effect, but when injected intraäbdominally was fatal in doses of from 0.1 to 0.2 cc. Within ½ hour after the injection of the toxin, the coat became rough, the mice assumed a crouching position, gradually became comatose, and died within 24 hours. When the drug was administered orally in doses of 10, 15, or 25 mg at 1, 2, 5, and 24 hours following injection of staphylococcal toxin, 386, or 87.7%, of 440 mice survived, while none of 110 controls lived (Table I). The treated mice showed the same symptoms as the controls until the drug became effective, which usually occurred after the third dose. Then, they recovered within 48 hours. It was also observed that

* Prontosil was named "Neoprontosil" when approved by the Council on Pharmacy and Chemistry of the American Medical Association. Neoprontosil was supplied by the Winthrop Chemical Company.

2 Dolman, C. E., *Canadian Public Health J.*, 1932, **23**, 125.

TABLE I.

Therapeutic Effect of Neoprontosil Administered Orally to Mice Injected Intraäbdominally with Bacterial Toxins.

Toxin	No. of mice injected	M.L.D. of toxin, cc	Amt Neoprontosil administered in mg	Treated Mice			Control Mice			Difference* / S.E.D.M.	Odds to 1 against difference being result of chance
				No. injected	No. survived	% survived	No. injected	No. survived	% survived		
Staphylococcal	550	0.1	40 60 100	440	386	87.7	110	0	0.0	18.0	$\rightarrow \infty$
Streptococcal	110	1.5	100	80	62	77.5	30	2	6.7	6.7	$> 5 \times 10^8$
Gonococcal	80	0.2	100	60	3	5.0	20	0	0.0	1.0	2.15
Clostridium welchii	140	0.08	125	100	84	84.0	40	4	10.0	8.2	$\rightarrow \infty$
Clostridium botulinum	80	0.15	100	60	52	86.7	20	1	5.0	6.7	$> 5 \times 10^8$

*Difference ÷ standard error of the difference of the means.

TABLE II.

Therapeutic Effect of Neoprontosil Administered to Mice Injected Intraäbdominally with Bacterial Toxins.*

Toxin	No. of mice injected	M.L.D. of toxin, cc	Dose of neoprontosil in mg	Treated Mice			Control Mice		
				No. injected	No. survived	% survived	No. injected	No. survived	% survived
Subcutaneous Administration of Drug.									
Staphylococcal	75	.1	75	60	0	0.0	15	0	0
Gonococcal	75	.2	75	60	1	1.6	15	0	0
Clostridium welchii	75	.08	75	60	4	6.6	15	3	20
Intraäbdominal Administration of Drug.									
Staphylococcal	75	.1	100	60	2	3.3	15	0	0
Gonococcal	84	.2	100	64	5	7.6	20	0	0
Clostridium welchii	75	.08	100	60	5	8.3	15	2	13.3

*The negative results obtained from the injection of neoprontosil are without statistical significance.

25 mg of Neoprontosil given orally 2 hours prior to the toxin protected 43, or 71.7%, of 60 mice, while all of 20 controls died.

Streptococcal Toxin.† The toxin employed in this experiment was produced from a strain of hemolytic streptococci isolated from a patient with puerperal septicemia. The intraäbdominal injection of 1.5 cc of the toxin killed 28, or 93%, of 30 mice within 72 hours. Immediately after injection, the mice showed severe twitching of the muscles. This symptom, which may have been caused in part by the phenol used as a preservative in the toxin, subsided within an hour. The untreated mice became listless, assumed the usual crouching position, and died within 72 hours. Neoprontosil in doses of 25 mg given orally at 1, 2, 5, and 24 hours after the toxin prevented death in 62, or 77.5%, of 80 mice. The treated mice showed similar symptoms until after the last dose of Neoprontosil at 24 hours, when improvement was noted. Recovery occurred in from 4 to 5 days.

Gonococcal "Toxin" The preparation referred to as gonococcal "toxin" was a lyophilized and regenerated whole broth culture of the gonococcus containing no viable organisms. It was so concentrated that 0.2 cc consistently killed the mice within 24 hours. We[3] have previously reported that sulfanilamide affords a high degree of protection to mice injected with this toxin. Attempts to duplicate this work by the oral administration of Neoprontosil, however, have been unsuccessful. Doses of 25 mg were given 1, 2, 5, and 24 hours after the toxin. Only 3, or 5%, of 60 mice so treated survived the intraäbdominal injection of the toxin. After injection with the toxin, both the treated and untreated mice were listless, their coats became rough, and they remained in a crouching position until death. Diarrhœa usually developed shortly after inoculation, the temperature became subnormal, and the eyelids became stuck together with a mucopurulent exudate. The drug also failed to protect mice when administered intraäbdominally or subcutaneously (Table II).

Clostridium welchii Toxin. The toxin of *Clostridium welchii* in doses of 0.08 cc injected intraäbdominally or intramuscularly killed 36, or 90%, of 40 mice. An area of necrosis developed at the site of injection within from 48 to 60 hours. Extensive edema then developed in the skin and muscles of the abdominal wall. The mice were active until death, which occurred suddenly 5 or 6 days after injection. Autopsy findings in untreated mice were: generalized edema, subcutaneous hemorrhage, and focal areas of necrosis in the

† Puerperal septicemia toxin Rx 017471A was supplied by Parke, Davis & Company.

[3] Carpenter, C. M., Hawley, P. L., and Barbour, G. M., *Science*, 1938, **88**, 530.

kidneys and liver. Eighty-four of 100 mice treated with Neoprontosil, as described above, survived. A small brownish area always appeared at the site of injection in these mice. Neither necrosis nor edema occurred. An autopsy performed 2 weeks later revealed no gross pathological changes.

Clostridium botulinum Toxin.‡ Toxins of *Clostridium botulinum,* types A and B, were used. Doses of 0.15 cc of either type uniformly killed the control mice within 48 hours. Within 24 hours, the mice showed extreme muscular weakness. Control of head movements was impaired, because the muscles of the neck were particularly affected. Within 36 hours the mice were moribund, and death occurred within 48 hours. The oral administration of Neoprontosil prevented the death of 52, or 86.7%, of 60 mice (Table I). These mice showed some weakness and loss of muscular control, but responded noticeably to the drug within 36 hours and were fully recovered in 3 days.

Summary. Neoprontosil was administered orally, subcutaneously, intraäbdominally, or intramuscularly to a total of 740 mice which had received lethal doses of 5 bacterial toxins by intraäbdominal injection. Oral administration of the drug prevented death in 87.7% of 440 mice given staphylococcal toxin, 77.5% of 80 mice given streptococcal toxin, 84% of 100 mice given the toxin of *Clostridium welchii,* and 86.7% of 60 mice given the toxin of *Clostridium botulinum.* On the other hand, only 5% of 60 mice injected with gonococcal "toxin" survived. In all instances, the compound was ineffective when administered parenterally.

‡ Toxins of *Clostridium botulinum,* A 172 and B 161, were supplied by the Lederle Laboratories.

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Effect of Parenteral Liver Extract on the Blood Picture of New Born Rats.*

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Previous work[1, 2, 3] has demonstrated the essential similarity in the transition toward normal adult levels of the peripheral blood pictures of new born rats and of the human with macrocytic hyperchromic anemia treated with liver extract. The transition in both may be interpreted[3, 4, 5] as the result of the substitution of normoblastic erythropoiesis in the place of megaloblastic erythropoiesis. There is, therefore, the possibility that the administration of liver extract may accelerate this transition in new born rats.

A control series of observations on the blood picture changes in 288 normal rats from birth to 24 days of age has been compiled to serve as a basis of evaluation of the effect of such an experimental procedure (see Table 3, reference 3).

The degree of variability or scatter as expressed by the standard deviation is proportionally large and markedly broadens the limits of non-significance ($X \pm 3\sigma$). Inasmuch as the young do not grow at the same rate, it is to be expected that blood formation in the different individuals will proceed at different rates. Litter mates frequently showed as large variations and differences as among unrelated animals of the same age. Data on the state of hemoconcentration in these rats are not available. A larger daily population would have smoothed the curves and reduced the variations.

Because of these considerations it is emphasized that only means, which are not too sharply delineated, and trends may safely be recognized. A sample of the error of observation is indicated by the coefficients of variation derived from 10 determinations on one blood specimen (see Table I, reference 3).

Two approaches have been utilized in determining the effect of

* Acknowledgment is made of the generous advice and aid rendered by Dr. A. J. Carlson and Dr. G. E. Wakerlin.

1 Wintrobe, M. M., and Shumacher, H. B., *J. Clin. Invest.*, 1935, **14**, 837.

2 Smith, C., *J. Path. and Bact.*, 1932, **35**, 717.

3 Bruner, H. D., Van de Erve, J., and Carlson, A. J., *Am. J. Physiol.*, 1938, **124**, 620.

4 Jones, O. P., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 222.

5 Kato, K., *J. Lab. and Clin. Med.*, 1935, **20**, 1243.

liver extract on the blood picture of young rats: (I) The injection of liver extract into the mother rats before and during pregnancy, with examination of the blood of the young at intervals after birth. (II) The injection of liver extract directly into the young after birth, with examination of the blood at intervals after injection.

The liver extract used was a clinically active commercial extract (several different batches) formerly designated as 3 cc derived from 100 g of liver, and now as 10 units per 3 cc.† The rats used were of the same stock and fed the same diets as the control rats; further, most of the observations were carried out during the same period. The technical and hematological methods were as previously described[3] and frequent checks were made to assure that the error of observation remained within fixed limits.

(I) Liver extract in doses of 2.5 cc per kg body weight was injected intramuscularly into 19 female rats once per week for 1 to 4 weeks before mating and throughout pregnancy. This weekly quantity of extract was estimated, on a weight basis, to be approximately 10 to 15 times the total quantity required to induce remission in the average case of pernicious anemia.

The blood values of the 144 young of these injected rats were determined in 32 rats within 24 hours after birth and in 14 young on the third day after birth and every third day thereafter up to the twenty-fourth day of age. The young of a litter were distributed over the entire period as far as possible.

The means and the standard deviations (Table I) of the blood values grouped as to age were checked against the control data by the method of the standard error of the difference of two means. The mean values of the erythrocytes, hemoglobin, and hematocrit were not significantly different from the corresponding means except in the instance of the erythrocytes on the twelfth, fifteenth, and eighteenth days; the means of the hemoglobin and hematocrit also tended to be higher on these days. In view of the definite non-significance on the ninth and twenty-first days, the differences do not assume importance. The increased values might readily be explained by hemoconcentration. The mean values of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were not significantly different. The mean values of the reticulocytes, percent and absolute, were significantly different from the means of the control groups only on the sixth day. It will be noted that the control value for this day is out of line. The leucocyte values

† Supplied in part by courtesy of Dr. Guy W. Clark, Lederle Laboratories, Inc., Pearl River, N. Y.

TABLE I.

Means (X) and Standard Deviations (σ) of Blood Values of 144 Young Rats from Liver Extract Injected Mothers.

Age in Days	N*	R.B.C.*		Hb.*		Ht.*		M.C.V.*		M.C.Hb.*		M.C.Hb.C.*		Reticulocytes* %		Reticulocytes* Absolute		W.B.C.*	
		X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ
1	32	2.560	0.354	11.56	1.50	36.49	5.92	143.9	14.7	45.8	5.7	31.7	2.3	92.6	4.7	2.369	0.360	4.43	1.92
3	14	2.525	0.460	9.29	0.71	31.12	3.00	124.0	10.7	37.2	4.3	30.6	3.2	46.1	12.4	1.238	0.422	3.57	1.08
6	14	2.530	0.254	8.20	0.40	28.79	1.14	114.5	9.2	32.7	2.6	28.5	1.5	42.7	11.1	1.047	0.151	3.58	1.39
9	14	3.175	0.320	8.93	0.81	30.71	2.78	97.3	5.4	28.2	3.2	29.0	2.3	38.6	8.8	1.242	0.238	3.84	0.93
12	14	3.745	0.342	8.72	0.81	29.73	2.63	80.0	5.8	23.4	2.0	29.3	1.8	26.1	5.5	0.956	0.199	4.13	0.73
15	14	4.315	0.411	8.92	0.76	30.00	3.05	69.9	5.7	20.8	1.3	29.7	1.5	21.0	5.0	0.903	0.217	4.25	1.87
18	14	4.575	0.597	8.41	1.10	27.70	4.31	61.4	7.1	18.5	3.2	30.5	1.7	20.4	4.4	0.922	0.205	4.34	0.93
21	14	4.715	0.565	8.26	0.83	26.68	2.32	57.2	5.2	17.7	2.2	31.0	1.4	20.4	7.0	0.978	0.466	4.41	1.00
24	14	5.140	0.425	9.36	1.27	31.79	4.56	62.0	6.8	18.2	2.2	29.5	1.6	23.2	6.7	1.190	0.627	3.95	1.07

*N = Number of rats; R.B.C. = Erythrocytes in millions per mm^3; Hb. = Hemoglobin in g per 100 cc blood; Ht. = % packed erythrocytes; M.C.V. = Mean corpuscular volume in cubic micra; M.C.Hb. = Mean corpuscular hemoglobin in gamma gamma; M.C.Hb.C. = Mean corpuscular hemoglobin concentration in %; Reticulocytes expressed in % erythrocytes and absolute in millions per mm^3; W.B.C. = Leucocytes in thousands per mm^3.

showed no significant differences. Only 1.2% of the 1,306 measurements were beyond the limits of the control group; 0.8% of the measurements were also located beyond the limit of the $X \pm 3\sigma$ for the injected series.

There was found, thus, a lack of consistent differences between the examined blood values of the experimental and control groups. While it is possible that some of the young of the injected mothers showed significant differences, the majority did not. Further, rather large differences were sometimes obtained between similar blood values of litter mates of the same age as in the control group.

It may be added that the injection of liver extract did not prevent the appearance, qualitatively, of the prepartum anemia of the mother rat as described by Mitchell and Miller[6] and by Van Donk, Feldman and Steenbock.[7]

(II) The liver extract used for injecting the young rats was diluted 5 times with distilled water and injected subcutaneously. Leakage was prevented by sealing the puncture with a stiff vaseline. The dose used was 5 cc per kg body weight of undiluted extract and was calculated to be approximately 20 to 25 times the remission inducing dose for the human pernicious anemia patient. A sterile saline solution containing 0.5% phenol was used for control injections.

Twenty-eight litters each of 8 or more young were used. For each 2 young rats injected with diluted liver extract, 1 litter mate was injected with salt solution to serve as control. Two litters were injected within 8 hours after birth, and similarly 2 litter groups were injected for each day after birth up to 14 days of age. Studies were made on 2 liver extract-injected rats and 1 control rat of the litter on the fourth, tenth, fifteenth and, if the litter were large enough, twenty-first days after injection, recording the blood values according to age. Of the 182 liver extract-injected young, 30 were examined after the twenty-fourth day and are not included. Since the values of the young of one age at the different intervals after injection are distributed indiscriminately among one another, the means and standard deviations of the whole distribution are presented in Table II.

The means of the erythrocyte values of the injected rats from the twelfth to the eighteenth days tended to be higher than the means of the controls but the difference was not significant nor was the in-

6 Mitchell, H. S., and Miller, L., *Am. J. Physiol.*, 1931, **98**, 311.

7 Van Donk, E. C., Feldman, H., and Steenbock, H., *Am. J. Physiol.*, 1934, **107**, 1934.

TABLE II.
Means (X) and Standard Deviations (σ) of Blood Values of 152 Liver Extract Injected Young Rats.

Age in Days	N*	R.B.C.*		Hb.*		Ht.*		M.C.V.*		M.C.Hb.*		M.C.Hb.C.*		Reticulocytes* %		Reticulocytes* Absolute		W.B.C.*	
		X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ
4	4	2.815	0.299	8.60	.25	30.00	0.36	107.8	12.3	30.9	3.9	28.7	1.1	33.3	4.4	0.940	.173	2.15	0.42
5	4	2.405	0.250	8.34	.33	27.69	0.39	116.4	12.4	35.1	4.1	30.2	0.6	29.5	2.7	0.955	.422	3.15	1.54
6	3	3.415	0.372	8.34	.14	28.83	0.78	89.5	7.3	25.0	2.7	29.7	1.5	30.0	3.1	0.982	.164	4.12	1.65
7	4	2.895	0.501	8.64	.25	30.25	1.64	107.5	7.6	30.7	5.3	28.6	1.2	24.4	2.7	0.700	.100	3.11	0.33
8	4	2.900	0.126	8.31	.23	28.88	0.85	99.3	3.9	28.7	1.3	29.0	1.1	31.0	11.4	0.895	.310	3.64	0.89
9	4	3.490	0.465	9.06	.76	30.78	0.85	89.5	9.9	26.3	1.9	29.5	2.3	31.9	5.0	1.131	.280	3.69	0.44
10	8	3.710	0.113	8.90	.35	30.50	1.02	82.4	3.4	24.6	1.3	29.4	0.9	33.8	7.9	1.252	.275	3.63	0.75
11	11	3.445	0.177	7.99	.49	27.93	1.11	81.4	3.2	23.2	1.7	28.6	1.3	26.8	5.9	0.916	.184	3.61	0.86
12	8	3.585	0.150	7.61	.56	25.78	2.87	72.3	8.8	27.1	3.4	29.5	1.3	26.9	3.6	0.968	.152	4.28	0.81
13	5	4.135	0.122	8.46	.50	28.50	0.87	69.1	3.8	20.5	1.0	29.7	0.8	16.6	2.9	0.682	.141	4.81	0.46
14	10	4.485	0.421	9.19	.86	31.05	3.15	69.3	4.0	20.6	1.2	29.6	1.5	21.8	4.6	0.971	.201	4.40	1.99
15	12	4.000	0.403	7.78	.50	26.64	1.53	67.4	4.4	19.5	1.2	29.2	1.0	19.5	3.0	0.769	.121	3.58	0.89
16	12	3.855	0.374	7.73	.48	25.79	1.86	67.6	4.9	20.2	1.0	29.9	1.3	15.0	3.6	0.570	.157	4.44	0.89
17	15	4.230	0.197	8.08	.67	26.37	2.81	62.5	7.5	19.0	1.7	31.0	2.2	19.7	5.9	0.838	.244	3.56	0.69
18	6	4.230	0.155	7.22	.33	23.59	0.85	55.8	4.7	17.1	1.6	30.7	1.3	21.0	1.7	0.889	.088	4.14	1.13
19	11	4.185	0.332	7.13	.54	24.39	1.43	58.5	1.3	17.3	2.0	29.3	1.6	17.9	3.7	0.750	.131	5.48	1.53
20	4	4.875	0.351	8.74	2.24	28.00	5.22	57.0	7.1	17.7	3.5	30.8	2.2	22.2	8.8	1.053	.351	4.28	1.49
21	6	4.600	0.196	8.03	.18	28.21	1.80	61.7	2.9	17.5	0.9	29.5	1.4	23.6	2.7	1.076	.051	4.61	0.49
22	12	4.905	0.531	7.91	.59	27.40	3.07	56.0	5.4	16.2	2.9	29.0	1.7	26.3	6.8	1.180	.328	3.40	0.78
23	9	5.595	0.714	9.06	.49	32.08	1.73	58.1	5.3	16.4	4.0	28.2	0.7	22.9	5.7	1.313	.479	4.39	1.85
24	4	5.415	0.806	9.04	.78	32.31	2.77	61.6	14.1	17.2	4.0	28.0	1.1	22.8	2.8	1.223	.148	4.75	1.47

*R.B.C. = Erythrocytes in millions per mm^3; Hb. = hemoglobin in g per 100 cc blood; Ht. = % packed erythrocytes; M.C.V. = Mean corpuscular volume in cubic micra; M.C.Hb. = Mean corpuscular hemoglobin in gamma gamma; M.C.Hb.C. = Mean corpuscular hemoglobin concentration in %; Reticulocytes expressed in % erythrocytes and absolute in millions per mm^3 W.B.C. = Leucocytes in thousands per mm^3; N = Number of rats.

flection maintained. A few isolated means of the other 8 blood values were statistically significant; the remainder were not. Thus, the great majority of the variations observed could have occurred by chance.

Comparison of the values of the 2 liver-injected rats with their litter mate control showed that the control value was located between the 2 corresponding values of the injected rats in 41%, 39%, and 42% of instances on the fourth, tenth and fifteenth days after injection respectively. This is approximately what should occur by chance. The blood values of the 30 injected rats between the twenty-fifth and thirtieth days continued the trends of the control group and were comparable to the litter control rats. Of the total of 1,368 determinations, only 0.95% were beyond the limits of $X \pm 3\sigma$ of the control group.

Figures 1 and 2 present graphically the most useful criterion, the mean corpuscular volumes of the erythrocytes, in estimating the transition changes in the blood of the experimental groups as compared to the control group of young rats. None of the means are statistically significant.

Growth in both experimental groups of rats as judged by the weight and crown-tailbase length was comparable to control data.

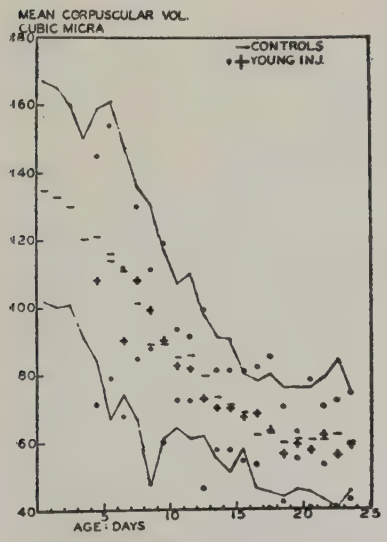

FIG. 1.

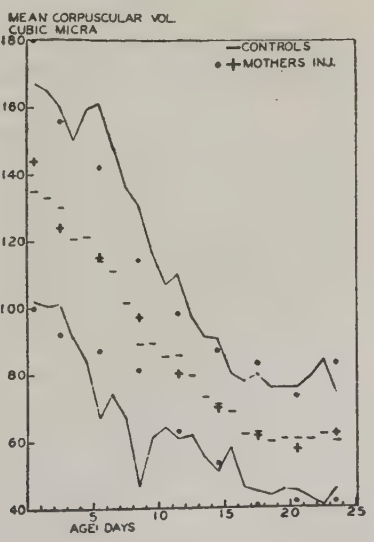

FIG. 2.

Figs. 1 and 2 illustrate the location of the means (+) and the $\pm 3\sigma$ (•) of the mean corpuscular volumes of the erythrocytes of the 2 experimental groups of young rats at indicated ages after birth in relation to the mean (—) $\pm 3\sigma$ (continuous line) of the control rats.

The lack of a marked acceleration of normoblastic erythropoiesis or of a lessened megaloblastic erythropoiesis is indicated especially by the not significantly different decline of the mean corpuscular volume, level of the reticulocytes, and increase of erythrocytes. Fitz-Hugh, Creskoff and Taylor[8] indicated the ineffectiveness of daily injections of liver extract in changing the reticulocyte count of 14 nursling rats. The injection of liver extract into the fetuses of 2 pregnant rats was likewise ineffective. Wintrobe, *et al.*,[9] found that liver extract injected into 3 pregnant rabbits produced no effect on the erythrocytes of fetuses extracted before term. Also, the injection of 0.15 cc of liver extract into the placentas of rabbit fetuses in one horn of the uterus did not alter the blood picture of those fetuses in comparison with the fetuses in the uninjected horn. These workers reported that extracts of fetal pig livers and of the placenta, even in the last third of pregnancy, were inactive. Wigodsky, *et al.*,[10] however, found fetal calf livers anti-anemically active. Wigodsky and Ivy[11] injected liver extract into pregnant rats from 2 to 14 days before delivery. They observed no significant differences between the values of the erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, or the mean red cell diameter of new born rats from normal and antenatally injected mothers. Their data indicate variations similar to those encountered above.

Briese and Higgins[12] have reported that the addition of ventriculin to the diet of pregnant rats resulted in a decrease of the mean diameter of the erythrocytes of the new born rats together with a shift of the Price-Jones curve to the left as measured from dry smears. The variability in the erythrocyte counts of normal new born led them to suggest that variations in the maternal stores of the anti-anemic principle may occur. Stasney and Higgins[13] have reported that the daily intraperitoneal injection of neutralized normal human gastric juice into pregnant rats for 12 days preceding parturition resulted in a reduction of the diameters and corpuscular volumes of the erythrocytes of the new born. Heated gastric juice

[8] Fitz-Hugh, T., Jr., Creskoff, A. J., and Taylor, H. B., *J. Clin. Invest.*, Proc., 1936, **15**, 468.

[9] Wintrobe, M. M., Kinsey, R. E., Blount, R. C., and Trager, W., *Am. J. Med. Sci.*, 1937, **193**, 449.

[10] Wigodsky, H. S., Richter, O., and Ivy, A. C., *Am. J. Physiol.*, Proc., 1938, **123**, 215.

[11] Wigodsky, H. S., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 787.

[12] Briese, E., and Higgins, G. M., *Anat. Rec.*, 1939, **73**, 105.

[13] Stasney, J., and Higgins, G. M., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 490.

produced no effect. Similar effects were obtained by Stasney, Higgins, and Mann[14] using the gastric juice obtained from a fundic pouch of a pig. Briese[15] reported that the blood picture of the new born of rats from mothers rendered anemic by daily inhalations of CCl_4 during pregnancy was more macrocytic than the blood of young from normal mothers.

The lack of significant changes probably are not due to administration of insufficient amounts nor to the mode of administration of liver extract. Connery and Goldwater[16] and Riddle and Sturgis[17] have shown that single massive doses are effective in inducing remission in human pernicious anemia. It is interesting to observe also that the liver preparation did not produce a reduction in erythrocytes or hemoglobin. This effect of liver has been reported by Marshall[18] after injection of liver into rats made polycythemic by cobalt. Adlersberg and Leiner[19] reported that anemia may be produced in normal rabbits by the administration of liver preparations both orally and parenterally; a powdered liver preparation given orally to dogs also produced an anemia.

Assuming that the rat requires the anti-pernicious anemia principle, the lack of effect of the administered liver extract in the young rats is similar to that observed in the human macrocytic hyperchromic anemia patient previously given optimal amounts of liver extract. It would appear that in the presence of adequate amounts of anti-anemia substance some other factor becomes the limiting agent in the mechanism of blood production.

Summary. 1. The transition from megaloblastic to normoblastic erythropoiesis appears to be reflected in data on the blood cell values of normal rats from birth to 24 days of age. 2. Blood cell values of young whose mothers were injected with liver extract before and during pregnancy were found to be not significantly different from control values at comparable ages after birth. 3. Blood cell values of young rats directly injected with liver extract up to the fourteenth day after birth were found not to be significantly different from control values at comparable ages after birth. 4. The young rat's reaction to the presence of excess anti-anemic principle resembles that of the human macrocytic hyperchromic anemia patient maintained on adequate amounts of liver extract.

14 Stasney, J., Higgins, G. M., and Mann, F. C., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 699.

15 Briese, E., *Am. J. Med. Sci.*, 1938, **195**, 787.

16 Connery, J. E., and Goldwater, L. J., *J. Lab. and Clin. Med.*, 1932, **17**, 1016.

17 Riddle, M. C., and Sturgis, C. C., *Am. J. Med. Sci.*, 1930, **180**, 1.

18 Marshall, L. H., *Am. J. Physiol.*, 1936, **114**, 194.

19 Adlersberg, D., and Leiner, G., *Z. f. d. ges. exper. Med.*, 1936, **98**, 398.

10642

Metabolism of Leucocytes in Ringer-Phosphate and in Serum.

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Ponder and MacLeod[1, 2] described the oxidative rate of leucocytes obtained from peritoneal exudates in rabbits, but no studies of glycolysis were made, and Ringer-phosphate solution was the only medium employed. In this communication are presented the results of studies of aerobic and anaerobic glycolysis of exudate leucocytes, and a simple method by which their respiration in serum may be measured is described.

Bakker[3] stated that exudate leucocytes respired at a low rate ($QO_2 = 0.4$) but the rate of aerobic glycolysis was high ($Q_G^{O_2} = 6$). Fleischmann and Kubowitz[4] reported rates 10 times as great ($QO_2 = 4$) for the respiration of similar cells, and figures for aerobic and anaerobic glycolysis of 11 and 21 respectively. Both authors used Ringer-phosphate solution as a medium. Fujita[5] using rat blood leucocytes suspended in citrated rat plasma reported a high respiratory rate ($QO_2 = 9$) and a low rate of aerobic glycolysis ($Q_G^{O_2} = 2$).

The experiments of Bakker and of Fleischmann and Kubowitz have been repeated and the results are here reported. The leucocytes of peritoneal exudates induced in the rabbit were suspended in Ringer-phosphate solution. The technic for obtaining the cells has been described.[1, 2] The cell suspension used contained about 70,000 cells per mm^3, and 2 cc of the suspension were sufficient to allow good measurements of the metabolic rate over a period of 3 hours.

Respiration was measured in Ringer-phosphate at a pH of 7.3, in an atmosphere of pure O_2. For aerobic glycolysis, the cells were suspended in Ringer-glucose-bicarbonate, and the measurements made after equilibration with 95% O_2 and 5% CO_2. For anaerobic glycolysis the cells were suspended in the same medium equilibrated with 95% N_2 and 5% CO_2. The results express the average metabolic activity during the first hour in 10 experiments.

QO_2	$Q_G^{O_2}$	$Q_G^{N_2}$
4.6	17	25

1 Ponder, E., and MacLeod, J., *J. Gen. Physiol.*, 1936-37, **20**, 267.

2 Ponder, E., and MacLeod, J., *Am. J. Physiol.*, 1938, **123**, 420.

3 Bakker, G., *Klin. Woch.*, 1927, **6**, 252.

4 Fleischmann, W., and Kubowitz, F., *Biochem. Z.*, 1927, **181**, 395.

5 Fujita, A., *Klin. Woch.*, 1928, **7**, 897.

These figures are slightly higher than those of Fleischmann and Kubowitz, but are in the same range. We cannot confirm the low rate of respiration reported by Bakker. The cause of the abnormally high rate of aerobic glycolysis observed is obscure. It is possible that the effectiveness of respiration in preventing aerobic glycolysis has been damaged in these cells, either by the methods used in obtaining them, or by the use of Ringer-phosphate solution as the suspension medium.

The effect of serum on the respiration of exudate leucocytes has been investigated. Normal serum cannot be used in the presence of KOH because of the marked pH changes due to the loss of CO_2. It is possible to modify rabbit serum so that the bicarbonate content is reduced and the resulting pH change in the presence of KOH is relatively small. The method of preparation is as follows: 15 cc of serum are titrated with N/10 HCl until the pH is about 6.4. In the case of normal rabbit serum which has been exposed to the air for some time from 1.5 to 2 cc of N/10 HCl are required. The acidified serum is then thoroughly evacuated until the pH reaches 7.3. Measured quantities are then transferred to the Warburg vessel, the cells added, and respiration measured in the conventional manner in an atmosphere of O_2, KOH being used to absorb CO_2. In this way, special apparatus is not necessary.*

In 12 experiments the respiration of exudate leucocytes has been measured in neutralized serum. In all the respiration was greater than in Ringer-phosphate. This increase varied from 35 to 70% with a mean of 50%. The mean QO_2 in neutralized serum is 7 as compared to a QO_2 of 4.6 in Ringer-phosphate. Furthermore, maximum respiratory activity is maintained longer in serum than in Ringer-phosphate. The serum alone consumes sufficient O_2 to account for not more than 15% of the increase.

In several control experiments the change in pH of the neutralized serum in the manometer in the presence of KOH for a period of time equivalent to the usual duration of an experiment has been measured. The changes were from 0.2-0.3 toward the alkaline side (7.3-7.6). In the case of exudate leucocytes where aerobic glycolysis is of considerable magnitude this change of pH is offset by acid production The pH of the cell-serum system after 3 hours in the manometer in presence of KOH falls from 7.3 to approximately 6.9.

* Dr. C. O. Warren, Jr., has applied the neutralized serum technic to the respiration of bone marrow and compared the results obtained with those of experiments done in untreated serum in the Dixon-Keilin manometer; he found no appreciable difference in the QO_2 values in both systems though both showed a marked increase (50%) over that of Ringer-phosphate (private communication).

Summary. The rates of respiration and aerobic glycolysis of exudate leucocytes reported by Fleischmann and Kubowitz have been confirmed. A method of measuring the respiration of leucocytes in serum is described. The respiratory rate of leucocytes in serum is greater than the rate in Ringer-phosphate solution.†

10643 P

Effects of Female Sex Hormones in Young Opossums.

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In a recent communication[1] the writer described the effects of the male hormone, testosterone propionate, on the developing genital tracts in pouch young of the American opossum, *Didelphys virginiana.* This hormone has a dual action, stimulating the development of both male and female structures in animals of either genetic sex. However, male parts are uniformly better developed in male subjects, and conversely, female structures show greater growth in females. These results illustrate clearly the interaction of hormones and genotype in development. The present report deals with a similar experiment, in which the female hormones estradiol dipropionate and estrone were administered from the 4th to the 18th day of pouch life.

The group treated with estradiol dipropionate consisted of a litter of 12 young (8 males, 4 females) which received a total dose averaging 20-25 gamma daily. Externally no effects were observed until the ninth day, when several individuals showed a distention of the abdomen, and a pronounced enlargement of the phallus, of a different morphological character from that induced by male hormones. Later the abdominal condition had become worse, and 2 individuals appearing moribund, were sacrificed on the 12th day. From day to day others were preserved for the same cause, the last on the 18th day.

At first the symptoms were ascribed to toxicity of the solution; however, autopsy showed a greatly distended bladder, enormous sac-

† We are greatly indebted to Dr. E. Shorr of the New York Hospital for much valuable advice concerning the use of serum.

1 Burns, R. K., Jr., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, —

like dilatations of both Wolffian ducts and ureters, and a similar condition of the pelvis of the metanephros. Both meso- and metanephros showed great damage, probably of a mechanical origin, primarily. These animals were apparently dying of a urinary retention, and the cause was found in the condition of urethra and urinogenital sinus, in which an extreme cornification had resulted in complete obstruction of the outlets of the urinary system. With this condition in mind, the effects upon the various parts of the urinogenital apparatus will be summarized.

Wolffian ducts are extremely dilated in both sexes, particularly in the posterior region adjacent to the obstructed urinogenital sinus. This dilation is apparently mechanical in nature, occurs in both sexes, and is not attributed to hormone action.

Müllerian ducts are markedly increased in size in both sexes, but show an important defect in the absence of the posterior region. The ducts end blindly in the wall of the dilated posterior sac of the Wolffian duct. Apparently the tremendous expansion of this sac has prevented the posterior tip of the growing Müllerian duct from establishing a connection with the urinogenital sinus, and the defect cannot be attributed specifically to hormone treatment. The age of these specimens naturally precludes a growth of the female ducts comparable to that found in the previous experiment.

The urinogenital sinus, as stated, shows an extreme cornification of the mucosa, extending to the sinus horns (which contribute to the lateral vaginæ) and into the urethral region of the bladder including the ureteral papillæ. The transition to bladder and Wolffian duct epithelium is abrupt. The lumen of the sinus is reduced to very small caliber by excessive delamination of cornified layers, and completely obstructed by debris. Prostatic glands are lacking in both sexes, although they should be present in older males of the group.

Phallus and urinogenital meatus are extremely enlarged and hyperemic. The form of the organ and its posture—flat against the body wall—are in marked contrast to the normal male organ and the erect posture induced by male hormone treatment. The modified organs appear identical in both sexes.

Pouch and scrotum are apparently unaffected, which agrees with their failure to react to the androgen, as reported.

The gonads are not changed so far as histological type is concerned, but are reduced in size. In the case of testes this reduction is extreme. The sex cords are small and shrunken, imbedded in a dense stroma. This amount of estradiol dipropionate appears to be strongly inhibitory to testis growth.

Another litter of 8 young receiving estrone treatment shows the same modifications induced by estradiol dipropionate in lesser degree.

Summary. Estradiol dipropionate and estrone have only a gynogenic action on the genital tracts of young opossums of either sex, contrasting in this respect with the male hormone testosterone propionate which exerts a dual effect. The most striking effects are exerted on the phallus and the epithelium of the urinogenital sinus. Müllerian ducts, although very immature, are definitely enlarged. Prostatic outgrowths are suppressed.

10644

A Study of the Fever-Producing Principle in the Typhoid Vaccine.*

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For a quarter of a century typhoid vaccine has been used clinically in what has been called non-specific protein-therapy.[1] The symptoms provoked by the intravenous injection of typhoid vaccine are shown in experiment 1 in Table I. They are fever, shivering (chills), leucopenia, and gastro-intestinal disturbances, such as vomiting and diarrhea. These are in the main, the symptoms mentioned by Hektoen[2] and Cecil[3] as following the intravenous injection of foreign proteins. These symptoms are also paralleled by those elicited in the "pyrogenic reaction" following the intravenous infusion of "pyrogenic" fluids and "pyrogenic" inulin as evinced in experiments 2 and 3 in the table.

The "pyrogen" of infusion-fluids was shown by Hort and Penfold[4] to pass through a Berkefeld candle. This was confirmed by Seibert,[5]

* We wish to thank Dr. Johannes H. Bauer of the Rockefeller Institute for instructions in the preparation and calibration of Elford-Bauer membranes used in this study.

1 Petersen, *The Newer Knowledge of Bacteriology and Immunology*, Jordan and Falk, The University of Chicago Press, 1928, p. 1086.

2 Hektoen, *J. A. M. A.*, 1935, **105**, 1765.

3 Cecil, *Ibid.*, 1935, **105**, 1846.

4 Hort and Penfold, *Brit. Med. J.*, 1911, **2**, 1589.

5 Seibert, *Am. J. Physiol.*, 1923, **67**, 90.

TABLE I.

Exp. No.	Material injected intravenously	Wt of dog, kg	Post-injection symptomatology						
			0 time	45 min	1½ hr	2 hr	3 hr	4 hr	5 hr
1	Whole typhoid vaccine, 1 cc	12.5	t—100.2 w—18.4	t—102.6 w—6.4	t—102.6 Shivering	t—103.2 Emesis Defecation	t—103.4 Shivering	t—102.8 Depressed	t—101.8
2	6% "pyrogenic" dextrose soln., 400 cc	11	t—101.6 w—17	t—102.8 w—2.3	t—104.5 Emesis	t—106 Defecation Shivering	t—104.5	t—103.2	t—102.4
3	10% "reactive" inulin 25 cc	17	t—101.8 w—16.5	t—104.4 w—8.8	t—104.8	t—105.3 Emesis Defecation	t—105.3	t—104.2	t—103.2
4	Berkefeld filtrate of typhoid vaccine, 1 cc	13	t—100.6 w—20.2	t—101.6 w—2.1 Shivering	t—104 Bloody stool	t—104.6 Emesis (bile) Bloody stool	t—104.4 Depressed	t—103.8	t—102
5	Typhoid broth, 1 cc	11.7	t—101.4 w—15	t—102 w—2.75 Emesis	t—103 Shivering Defecation	t—103.6 Shivering	t—104 Emesis	t—103.6 Depressed	t—102.8
6	Berkefeld filtrate of typhoid vaccine refiltered through 200 sec Zsigmondy, 18 cc	16	t—101.4 w—20	t—101.2 w—18	t—101.5 No symptoms	t—100.8	t—101	t—100.6	
7	Berkefeld filtrate of typhoid vaccine refiltered through Seitz, 30 cc	15	t—101.6 w—22.7	t—101.6 w—23	t—101 No symptoms	t—100.2	t—101.2		
8	Typhoid broth filtered through Seitz, 500 cc	14.5	t—101.4 w—12.6	t—101.6 w—15.5	t—101 No symptoms	t—101.2	t—101.2		

t—Rectal temperature, °F.
w—Leucocytes in thousands.

Banks,[6] and Co Tui, Schrift, McCloskey and Yates.[7, 8, 9] These last authors also showed that this "pyrogenic" principle was held back by a 200-second Zsigmondy filter as well as by an especially prepared asbestos filter pad of the type of Seitz Serum No. 3.

The purpose of the present study was to determine whether in addition to this symptomatic similarity, the fever-producing principle in typhoid vaccine exhibited filtration-characteristics similar to "pyrogen." The Berkefeld filtrate of broth in which *B. typhosus* had been grown for 48 hours (hereafter called typhoid broth) was likewise investigated. The test animal used in this study, as in our previous work, was the dog.

Berkefeld Filtration. Experiment 4 shows the clinical response to 1 cc of a Berkefeld filtrate of the typhoid vaccine used in Experiment 1. It will be seen that the clinical response is even more marked than in the case of the whole vaccine, the leucopenia being more profound, and the thermal rise higher. The gastro-intestinal disturbances were also more severe, with bile in the vomitus, and blood in the diarrheic stool.

Experiment 5 shows that typhoid broth, which, it will be remembered, is a Berkefeld filtrate, also contains the fever-producing principle.

It is thus clear that the fever-producing principle associated with *B. typhosus* is not bound to the bacterial bodies but may be separated from them by Berkefeld filtration. It may also be mentioned that the supernatant cell-free liquor of the centrifuged vaccine is likewise a potent fever-producer.

Membrane Filtration. Experiment 6 shows that the fever-producing principle in the Berkefeld filtrate of typhoid vaccine, like the "pyrogen" of inulin and infusion-fluids is held back by a 200-second Zsigmondy filter. That in typhoid broth, however, is not so removed. This may be explained on the basis of the increased permeability of collodion membranes caused by the presence of broth. This increased permeability was shown by Galloway and Elford[10] in the case of viruses. However, filtration through an isoporous membrane (Elford-Bauer), 100 mμ, removed the "pyrogenic" principle from the typhoid broth as well as from the typhoid-vaccine filtrate.

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[6] Banks, *Am. J. Clin. Path.*, 1934, **4**, 260.

[7] Co Tui, McCloskey, Schrift, and Yates, PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 297.

[8] Co Tui, Schrift, McCloskey, and Yates, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 227.

[9] Co Tui, McCloskey, Schrift, and Yates, *J. A. M. A.*, 1937, **109**, 250.

[10] Galloway and Elford, *Brit. J. Exp. Path.*, 1931, **12**, 407.

Asbestos Pad Filtration. Experiments 7 and 8 show that the fever-producing principle in both typhoid broth and the Berkefeld filtrate of typhoid vaccine, is removed by filtration through a Seitz serum No. 3 filter. It may also be mentioned that recent experiments in our laboratory show that another asbestos filter pad, the Ertel No. 0, is equally as efficient in the removal of pyrogen as Seitz. In this respect, too, it reacts similarly to the "pyrogen" in infusion-fluids and inulin.[8]

Conclusions. 1. The fever-producing principle in typhoid vaccine and in broth in which *B. typhosus* has grown for 48 hours is not removed by Berkefeld filtration. The principle is, therefore, not bound to the bacterial bodies.

2. The principle is removed by a 200-second Zsigmondy filter and is, therefore, of approximately the same size as the principle previously found in reactive inulin and infusion-fluids.

3. Like "pyrogen" found in inulin and in infusion-fluids, it is removed by filtration through asbestos pads of the types of Seitz and Ertel.

4. On the basis of the clinical response provoked by intravenous injection, and the filtration characteristics, it is submitted that the fever-producing principle associated with *B. typhosus* and the "pyrogen" found in infusion-fluids and inulin are closely related substances.

10645

Effect of Electrolyte Disturbance on Resistance to Histamine Poisoning in Rats.

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Following suprarenalectomy there is a profound disturbance in electrolyte metabolism,[1, 2, 3] It is possible to reproduce an analogous disturbance in normal animals by introducing large amounts of isotonic glucose intraperitoneally and subsequently withdrawing the fluid.[4] The effect of this procedure on resistance to histamine was determined.

Method: Twenty cc of isotonic glucose solution was introduced into the peritoneal cavity of each of a number of rats and 4 hours

1 Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1927, **81**, 86.

2 Loeb, R. F., Atschley, D. W., Benedict, E. M., and Leland, J., *J. Exp. Med.*, 1933, **57**, 775.

3 Zwemer, R. L., and Truszkowski, R., *Science*, 1936, **83**, 558.

4 Gilman, A., *Am. J. Physiol.*, 1934, **108**, 662.

TABLE I.
Electrolyte Disturbance Induced in Normal Wistar Rats, by Intraperitoneal Injections of Isotonic Glucose.

	I. Blood*		II. Peritoneal Fluid
	(Analysis before injection of glucose mg/100 cc)	4 hr after injection of glucose	(Analysis 4 hr after injection of 5.4% glucose)
Glucose (blood)	114	214	1.4%
Cl (serum)	342	321	292 mg/100 cc
Na (serum)	338	265	240 " "
K (serum)	22	55	18 " "
Non-protein nitrogen (blood)	34	58	32 " "
Total protein (serum)	6.1%	8%	0.15%

*A marked hemoconcentration was observed, the red cell count rising within 4 hr from 9 to 12 or 13 million per mm^3.

later the same quantities of fluid were withdrawn. Analyses of the constituents of the blood of such animals with intact suprarenals revealed a fall in the concentration of sodium and of chloride in serum, with a hemoconcentration, a rise in non-protein nitrogen, in total proteins and in the concentration of potassium. The decrease in concentration of serum sodium chloride apparently was due to diffusion of this substance into the peritoneal fluid (Table I). Immediately after withdrawal of the fluid the animals were injected with varying amounts of histamine.

Results: The minimal lethal dose of histamine for rats with intact suprarenals but in which an electrolyte disturbance was induced was 600 to 700 mg per kilo of body weight. Litter mates in which no electrolyte disturbance was induced survived from 1100 to 1200 mg histamine per kilo of body weight. (Table II.)

TABLE II.
Effect of Electrolyte Disturbance on Resistance to Histamine Poisoning in Rats.

No. rats	Histamine in mg per kg	Survived	Died
Rats given 20 cc of glucose intraperitoneally. Fluid withdrawn after 4 hours.			
2	600	2	0
9	700	1	8
5	800	0	5
8	1000	0	8
	Controls*		
4	1000	4	0
2	1100	0	2
5	1200	1	4
2	1300	0	2
2	1400	0	2
2	1600	0	2

*These controls represent only sample experiments from a large experience with the range of tolerance of rats of our stock to histamine.

It would appear that in part the disturbance in resistance following suprarenalectomy may be due to the disturbance in electrolytes resulting from withdrawal of the cortical hormone. However, this is only one factor, for administration of salt to suprarenalectomized rats will raise the resistance slightly but not to a degree comparable to that obtained with injections of suprarenal cortical hormone. Furthermore, suprarenalectomized rats are killed by an amount of histamine (100 to 200 mg per kilo of body weight) approximately one-sixth to one-tenth the M.L.D. for normal rats.

It is of interest to compare these results with the abnormalities which develop in humans suffering from similar electrolyte disturbances, as occurs in Addison's disease, and in heat cramps.[5]

Summary. The production in normal rats of an electrolyte disturbance analogous to that observed after suprarenalectomy is followed by a marked drop in resistance to histamine.

10646

Adult Phosphatase Levels in Prepubertal Rhesus Prostate Tissue after Testosterone Propionate.

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An "acid" phosphatase with optimum activity at about pH 5 is present in *adult* human prostate gland and seminal fluid[1] in concentrations greater (500-2,000 units/g fresh prostate tissue, in our series[2, 3]) than the phosphatase activity of any other human tissue. *Prepubertal* prostate gland, on the other hand, contains less than 5 units of "acid" phosphatase activity per g fresh tissue.[3, 4] An intermediate value of 73 units was found in the prostate gland of a 13-year-old boy.[3]

This correlation in man between sexual maturity and the "acid" phosphatase activity of prostate tissue suggested the possibility that stimulation of the prepubertal prostate gland by injection of testo-

5 Dill, D. B., *Life, Heat, and Altitude,* Harvard Press, 1938.

1 Kutscher, W., and Wolbergs, H., *Z. f. physiol. Chem.,* 1935, **236**, 237.

2 Gutman, E. B., Sproul, E. E., and Gutman, A. B., *Am. J. Cancer,* 1936, **28**, 485.

3 Gutman, A. B., and Gutman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 529.

4 Moore, R. A., and Hanzel, R. F., *Arch. Path.,* 1936, **22**, 41.

TABLE I.

Phosphatase Activity of Prostate Tissue of the Sexually Mature and Immature Rhesus Monkey; and of Immature Animals After Injection of Testosterone Propionate or of Estradiol Benzoate.

pH 4.9: M/200 monophenylphosphate substrate; M/10 citrate buffer; 37°C; 1 hr.
pH 9.0; M/200 " " M/20 Na veronal buffer; 37°C; 1 hr.

No.	Status	Wt, kg	Treatment	Wt both lobes prostate, g	Phosphatase Activity at pH 4.9 (units/g fresh prostate tissue)	Phosphatase Activity at pH 9.0 (units/g fresh prostate tissue)	Phosphatase Activity at pH 4.9 (units/whole prostate gland)	Phosphatase Activity at pH 9.0 (units/whole prostate gland)
1	Mature	10	Control	3.6	1,134	102	4,080	367
2	"	9.4	"	3.5	573	60	2,001	210
3	"	*6.0	"	3.2	356	22.5	1,140	72
4	Immature	3.5	"	0.7	1.2	1.2	0.8	0.8
5	"	3.5	"	0.6	2.8	—	1.5	—
6	"	4.0	"	0.3	4.8	0.4	1.4	0.1
7	"	4.0	Testosterone propionate (185 mg in 18 days)	2.0	975	30.5	1,950	61
8	"	2.9	Testosterone propionate (150 mg in 13 days)	1.1	779	14.8	858	16.3
9	"	2.1	Estradiol benzoate (6,000 rat units)	0.6	11.5	2.4	6.9	1.4

*Tuberculous animal.

sterone propionate would result in the precocious development of adult "acid" phosphatase levels. Rhesus monkeys were employed to investigate this possibility since, as indicated by Wolbergs,[5, 3] only in the monkey has the "acid" phosphatase activity of the prostate gland been found to be of the same order of magnitude as in man.

Methods. Experimental animals No. 7 and No. 8 (Table I) were injected daily (except Sunday) with 12.5 mg testosterone propionate, sacrificed after 18 and 13 days respectively; No. 9 received 500 Rat Units of estradiol benzoate in oil daily for 12 days.* Typical external evidences of the effects of these agents[6, 7] developed within the period of treatment. While the number of experimental animals is, of course, too small for definitive conclusions, the striking changes observed are thought to be significant.

Aqueous tissue extracts were prepared in the dilutions indicated elsewhere.[3] Tissue phosphatase activity at pH 9.0 was determined by the King and Armstrong method[8] and, in optimal dilutions, at pH 4.9 by the adaptation of that method previously outlined.[9] The results of tissue analyses are expressed in units, a unit being that degree of phosphatase activity which under the stated conditions of hydrolysis (Table I), will liberate one mg of phenol in one hour from the specified buffer—monophenylphosphate substrate solution. Serum phosphatase activity was determined by the Bodansky method.[10]

Results (Table I). In confirmation of Wolbergs,[5] at pH 4.9 we find marked phosphatase activity of prostate tissue of the *adult* Rhesus monkey, values of the same order of magnitude as in man. Unlike man, adult monkey prostate tissue exhibits appreciable phosphatase activity at pH 9.0, as in the rat.[3] In the *prepubertal* monkey, both "acid" and "alkaline" phosphatase activity of prostate tissue are negligible. Following treatment with testosterone propionate, the phosphatase activity of immature monkey prostate gland increases strikingly at pH 4.9 and at pH 9.0, reaching adult levels at the former and possibly also at the latter pH. Following treatment with estra-

5 Quoted by Kutscher, W., and Pany, J., *Z. f. physiol. Chem.*, 1938, **255**, 169.

* We are indebted to Ciba Corporation for testosterone propionate (Perandren) and to Dr. Schwenk of Schering Corporation for estradiol benzoate (Progynon B). We are further indebted to Dr. E. T. Engle for guidance and for histological sections of the prostates of the experimental animals.

6 Parkes, A. S., and Zuckerman, S., *Lancet*, 1935, **1**, 925.

7 Zuckerman, S., and Parkes, A. S., *Lancet*, 1936, **1**, 242.

8 King, E. J., and Armstrong, A. R., *Canad. M. A. J.*, 1934, **31**, 376.

9 Gutman, A. B., and Gutman, E. B., *J. Clin. Invest.*, 1938, **17**, 473.

10 Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

diol benzoate an equivocal increase in phosphatase activity was noted which cannot be interpreted as significant without further study.

The serum phosphatase activity at pH 9.0 was found to vary between 20 and 30 Bodansky units per 100 cc in immature Rhesus monkeys and was not significantly affected by injection of steroids; nor was the "acid" phosphatase activity of the serum,[11] which ranged between 2.5 and 3.6 units per 100 cc, affected thereby.

The phosphatase activity at pH 4.9 was found to be slight in the seminal vesicles (2.0-8.0 units/g fresh tissue), testis (4.0-5.6 units) and Cowper's glands (0.8 unit) of immature and mature Rhesus monkeys, with no significant difference in steroid-treated animals. The values obtained at pH 9.0 (0.6-3.9 units), (2.3-2.8 units) and (0.3 units) respectively, were also not different in the steroid-treated animals.

Discussion. The extraordinarily high "acid" phosphatase activity of prostate gland tissue in sexually-mature man and the monkey implies some as yet unknown prostatic function, effected by the enzyme. Within broad limits, the level of "acid" phosphatase activity of prostate tissue would appear to afford some measure of the capacity to exercise that function. In this sense, we interpret the effect of testosterone propionate in elevating the "acid" phosphatase activity of the prepubertal monkey prostate gland to adult levels as physiological evidence of a transformation in the direction of sexual maturity. This evidence is in harmony with the similar interpretation of concurrent morphological changes.[7] It will be noted (Table I) that estradiol benzoate, which acts chiefly upon the fibromuscular elements of the immature monkey prostate gland,[6] did not evoke a significant rise in "acid" phosphatase activity of prostate tissue; whereas a marked increase followed injection of testosterone propionate, which stimulates the development of the glandular epithelium.[7]

In our calculations (Table I), we conformed to common usage in regarding the prostate gland of the Rhesus monkey as composed of 2 lobes. The caudal lobe of the mature or stimulated gland, however, contains much more "acid" phosphatase than the cranial lobe: the ratio, *"acid" phosphatase activity per g caudal lobe/"acid" phosphatase activity per g cranial lobe* being 704/33, 2,270/124 and 1,357/86 in animals Nos. 3, 7, and 8, respectively.† In fact, such phosphatase

[11] Gutman, A. B., and Gutman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 470.

† The respective ratios at pH 9.0 were: 294/15.8, 53/10.3, and 22/6. In animal No. 9, however, the ratio at pH 4.9 was 9.6/19.9, at pH 9.0 2.9/2.5. Because of the small size of the cranial lobe in this animal, and the single observation, we are unable to say whether or not this deviation is significant.

activity as was found in the cranial lobe may well be due to reflux of caudal lobe secretion. The unequal enzyme distribution in the Rhesus gland suggests that the cranial lobe, if it is part of the true prostate gland, differs in function from the caudal lobe; as indicated also by differences in morphology and by van Wagenen's observations on the "coagulating" power of the cranial but not of the caudal lobe.[12]

While the function of the "acid" phosphatase of the mature prostate gland in man and in the monkey is not known, it would appear that suitable substrates are present in the seminal fluid (Ivanov,[13] and others) and that the slightly acid reaction of the vaginal secretion affords a favorable medium for its activity.

Summary. As in man, the prostate gland of the mature Rhesus monkey contains high concentrations of an "acid" phosphatase, whereas the prepubertal monkey prostate is virtually devoid of this enzyme. It is shown that testosterone propionate causes a several hundred-fold increase in "acid" phosphatase activity of the prepubertal monkey prostate gland to adult levels. It is inferred that in prepubertal man, so treated, an analogous increase occurs. In the monkey, "acid" prostate phosphatase is elaborated chiefly or solely in the caudal lobe.

10647

Combined Immuno and Chemotherapy of Pneumococcus Rat Infections.

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Fleming[1] has shown that type specific antipneumococcus serum increases the antibacterial action of sulfapyridine *in vitro* against pneumococci. McIntosh and Whitby[2] have shown, moreover, that sulfapyridine does not stimulate phagocytosis or antibody production against pneumococci, but suppresses their growth to a point that biological defense can become effective. Subsequently, we[3] showed that a small dose of broadly acting non-type-specific antipneumococc-

12 van Wagenen, G., *Anat. Rec.*, 1936, **66**, 411.

13 Ivanov, I. I., *Chem. Abstr.*, 1938, **32**, 2584.

1 Fleming, A., *Lancet*, 1939, **2**, 74.

2 McIntosh, J., and Whitby, L. E. H., *Lancet*, 1939, **1**, 431.

3 Powell, H. M., and Jamieson, W. A., *J. Immunology*, 1939, **36**, 459.

TABLE I.
Single Dose Antigen and Chemotherapy of Pneumococcus Infected Rats.
Type and Therapy of Pneumococcus Rat Infections.

Infecting Dose of Pneumococci, cc	I										II									
	Antigen 1	2	5	L	Sul + 1	" + 2	" + 5	" + L	Sul	Controls	Antigen 1	2	5	L	Sul + 1	" + 2	" + 5	" + L	Sul	Controls
10–1	1	1	2	1	7	4	5	6	1	1	1	1	3	1	1	1	S*	1	2	1
10–2	2	2	S	5	S	S	S	S	1	1	2	1	5	2	3	1	7	2	4	2
10–3	3	2	2	2	S	S	S	S	3	2	1	2	5	1	3	3	S*	4	4	1
10–4	3	1	S	S	S	4	S	S	S	1	3	1	5	2	3	3	5	2	4	2
10–5	S	S	S	5	S	3	S	S	1	2	S	1	S	S	3	3	S	4	2	2
10–6	S	2	S	S	S	S	S	6	3	2	3	2	S	5	S	3	S	S	S	2
10–7										S	S	4	S	S	S	4	S	S	S	3
10–8																				S
10–9																				

	V										VIII									
10–1	1	1	1	1	2	3	1	1	2	1	3	1	1	1	1	1	1	1	2	1
10–2	1	1	1	1	2	4	2	1	2	1	2	3	1	2	3	S	7	4	2	1
10–3	1	1	1	1	3	5	2	1	2	1	3	2	1	4	S	2	S	S	2	2
10–4	3	1	2	2	S	S	2	2	2	2	3	3	2	4	S	S	2	S	S	2
10–5	7	2	1	2	3	3	S	2	2	1	S	S	2	S	S	S	S	2	2	2
10–6	2	1	2	2	S	2	2	2	3	2	S	2	2	4	S	S	S	4	3	2
10–7	2	2	2	4	S	S	S	S	3	2										S
10–8	S	2	S	S	3	S	S	2	2	2										
10–9										S										

Legend: (1) Antigens 1, 2, and 5 made respectively from pneumococcus cultures DRI, type II and type V; Antigen L made from same cultures pooled. Sul = sulfapyridine.

(2) Numbers refer to day of death, after infection, of each rat; S = survival 7 days.

*These rats at termination of test were in poor condition and died of pneumococcus peritonitis two days later.

cus serum, of itself only partially effective, becomes highly effective when fortified with a single partially effective dose of sulfapyridine. This enhanced effectiveness was demonstrated in rat infections with pneumococci of 6 different types, and simplification of treatment was attained through use of a single broad antiserum.

Maclean, Rogers, and Fleming[4] have recently shown the importance not alone of passively introduced pneumococcus antibody but also antibody actively incited by pneumococcus vaccine in the combined immuno and chemotherapy of pneumococcus infection in mice and rabbits. These authors have made a strong case for combined

[4] Maclean, L. H., Rogers, K. B., and Fleming, A., *Lancet*, 1939, **1**, 562.

use of vaccine and sulfapyridine in treatment of pneumonia. They considered the utilization of 30 types of vaccine.

Inasmuch as we have used pneumococcus vaccine some of which has given broad effects in rabbits,[3, 5] it became of interest to test the combined therapeutic effectiveness of the vaccine and sulfapyridine directly in different types of pneumococcus rat infections. This report gives the results of these tests in a comparative way.

The pneumococcus cultures and vaccines used have been described heretofore.[3, 5] Vaccines made from certain cultures, namely, DRI, type II, and type V, had been found to incite the broadest antibodies in rabbits, hence vaccines of these separate and combined types were used in the experiments herein described. Since exposure of the vaccine to 37°C for a week appeared to enhance immunizing properties against heterologous type infections, this procedure was adhered to in preparation of these vaccines which always appeared preponderately gram negative. Preparation of somewhat similar pneumococcus vaccine almost 30 years ago by Rosenow[6] and Rosenow and Hektoen[7] should be mentioned.

Rats of 90-100 g weight were injected intraperitoneally with decimal dilutions of pneumococcus cultures I, II, V, and VIII. Separate groups of these were given the following respective treatments promptly after infection: (a) 1 cc pneumococcus vaccine subcutaneously; (b) 25 mg sulfapyridine orally; (c) treatment (a) and (b) together; (d) no treatment and kept for controls. Treated and control rats were observed for 7 days, and except in a few instances the survivors were discarded at this time. The results of these tests are shown in Table I and indicate that:

1. Pneumococcal antigen in a single dose exerts a definite therapeutic effect evidenced by prolongation of life or complete survival of pneumococcus infected rats. This effect is both homologous and heterologous as to type when certain culture antigens are used.

2. Enhanced therapeutic effects are obtained by combined use of one dose each of antigen and sulfapyridine.

3. These results verify and extend those of Maclean, Rogers, and Fleming, and simplify the vaccine needs in that a single antigen instead of 30 may suffice.

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5 Powell, H. M., and Jamieson, W. A., *Science*, 1939, **89**, 392.

6 Rosenow, E. C., *J. A. M. A.*, 1910, **54**, 1943.

7 Rosenow, E. C., and Hektoen, L., *J. A. M. A.*, 1913, **61**, 2203.

10648

Recovery of Pregnandiol in Urine of Men Treated with Progesterone.*

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Pregnandiol, first found in the urine of pregnancy by Marrian,[1] was identified and its chemical formula established by Butenandt.[2, 3] Following the isolation and artificial preparation of progesterone, it seemed probable that pregnandiol was a reduction product of the corpus luteum hormone[4] formed in the organism during pregnancy. At this time it had not yet been detected in the urine of males or non-pregnant females.

Browne and Venning[5, 6, 7] not only found large amounts of pregnandiol in the form of sodium pregnandiol glucuronidate in the urine of pregnancy but they also were able to obtain from 2 to 8 mg, calculated as free pregnandiol, from 24-hour specimens taken during the last half of the menstrual cycle in normal women. This work has been extensively confirmed in our laboratory and elsewhere.[8, 9] Consequently, pregnandiol is being used as a diagnostic aid in determining the presence and degree of corpus luteum activity in the female.

To show further the relationship between corpus luteum activity and pregnandiol excretion, Venning and Browne injected progesterone into women in whom there was a reasonable certainty that no corpus luteum activity existed, and recovered appreciable amounts of sodium pregnandiol glucuronidate.[7] In 2 cases, however, after

* Aided by a grant from the Rockefeller Foundation, and from the National Committee of Maternal Health.

† Fellow of the Rockefeller Foundation.

1 Marrian, G. F., *Biochem. J.*, 1929, **23**, 1090.

2 Butenandt, A., *Ber. Dtsch. Chem. Ges.*, 1930, **63**, 659.

3 Butenandt, A., *Ber. Dtsch. Chem. Ges.*, 1931, **64**, 2529.

4 Butenandt, A., and Mamoli, L., *Ber. Dtsch. Chem. Ges.*, 1934, **67**, 1899.

5 Venning, E. H., and Browne, J. S. L., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 792.

6 Browne, J. S. L., Henry, J. S., and Venning, E. H., *J. Clin. Invest.*, 1937, **16**, 678.

7 Venning, E. H., and Browne, J. S. L., *Endocrin.*, 1937, **21**, 711.

8 Wilson, R. B., Randall, L. M., and Osterberg, A. E., *Am. J. Obs. and Gyn.*, 1939, **37**, 59.

9 Stover, R. F., and Pratt, J. P., *Endocrin.*, 1939, **24**, 29.

TABLE I.

Patient	Sex	Age	Disease	Date	Progesterone injected mg	Pregnandiol recovered (computed)
G.W.	M	36	Addison's	2-6	30	Not done
				2-7	30	" "
				2-8	30	Pooled
				2-9	30	specimen
				2-10	30	Avg
				2-11	30	8.3 mg
				2-12	30	Not done
				2-13	30	20.1 mg
				2-14	30	Not done
E.L.	F	56	"	3-10	0	0
				3-11	30	0
				3-12	30	1 mg
				3-13	30	4.1 mg
				3-14	30	Not done
				3-15	30	2.4 mg
				3-16	30	3.8 "
				3-17	30	2.8 "
				3-18	0	1.6 "
				3-19	0	0
W.D.	M	22	"	3-7	0	0
				3-8	30	0
				3-9	30	3.5 mg
				3-10	30	Not done
				3-11	30	2.6 "
				3-12	30	2.5 "
				3-13	30	3.3 "
				3-14	30	2.5 "
				3-15	30	6.5 "
				3-16	0	0
L.B.	M	34	Normal	3-28	0	0
				3-29	30	5.3 mg
				3-30	30	6.2 "
				3-31	30	4.2 "
				4-1	0	3.8 "
				4-2	0	0

hysterectomy, no pregnandiol was excreted following the injection of 24 mg of progesterone.[10] They concluded that the uterus was necessary to metabolize progesterone to pregnandiol.

Hamblen confirmed this observation and found that pregnandiol, normally present during the luteal phase of the menstrual cycle, did not appear in the urine following curettage of the uterus. This indicated to him that the endometrium was in all probability necessary in this metabolic process.[11]

The most satisfactory method for determining the amount of

10 Browne, J. S. L., and Venning, E. H., *Am. J. Physiol.*, 1938, **123**, 209.

11 Hamblen, E. C., *Endocrin.*, 1939, **24**, 1.

pregnandiol in the urine is the gravimetric method of Venning,[12, 13] who obtained sodium pregnandiol glucuronidate by a butyl alcohol extraction method, the details of which are extensively described in her publications. We have determined sodium pregnandiol glucuronidate on all cases reported below by Venning's method, taking melting points to ascertain the purity of the product. The presence of sodium pregnandiol glucuronidate was also further demonstrated (1) by taking mixed melting points with the similar product from pregnancy urine, and (2) by hydrolysis in each instance of the glucuronidate to free pregnandiol. The hydrolysis was done by boiling one hour in a mixture of alcohol, 80%, concentrated HCl, 5%, and water, 15%. This was then diluted with water, extracted with ether and the pregnandiol was recrystallized several times from diluted ethyl alcohol. The melting points were between 235 and 237°C. The melting points of a mixture of this material with pregnandiol showed no depression.

Through the courtesy of Dr. Robert Loeb and Dr. Joseph Ferrabee we are permitted to report the results of pregnandiol determinations on 3 patients from the Presbyterian Hospital Medical Service who were suffering from Addison's disease. These three patients, one 56-year-old woman 11 years past the menopause, and two men, 22 and 36 years of age respectively, the latter having generalized tuberculosis, were given 30 mg of crystalline progesterone intramuscularly, daily.‡

Determinations on the urine of these patients disclosed that they all excreted pregnandiol in varying amounts during the period when they were receiving progesterone, but not at any other time. A control study was then done on a normal 34-year-old male, who received 30 mg daily for 3 days, and the same curve of excretion was observed.

The 36-year-old male suffering from Addison's disease and tuberculosis excreted an average of 8.2 mg a day on a 4-day pooled specimen. During one later 24-hour period, 20 mg of pregnandiol were excreted, or 67% of the injected progesterone. It is possible that the large amount excreted at this time had previously been retained in the tissues.

The other patients, on whom daily determinations were done, excreted from 1 to 6 mg daily. This is approximately the same quantity ordinarily excreted by normal women during the luteal

[12] Venning, E. H., *J. Biol. Chem.*, 1937, **119**, 473.

[13] Venning, E. H., *J. Biol. Chem.*, 1938, **126**, 2, 595.

‡ The progesterone used in this study was Progestin which was kindly furnished by Dr. Shaner of the Hoffmann-LaRoche Company.

phase of the menstrual cycle. The dosage of progesterone used in this series was probably greater than that reported by the previous workers.

Summary. Whereas previously, sodium pregnandiol glucuronidate was found only in pregnancy or in women with functioning endometriums, it has now been demonstrated in the urine of 2 men suffering with Addison's disease who were treated with 30 mg of progesterone daily, and in one normal young male with the same treatment.

10649

A Technic for the Study of Gastric Absorption in Man.*

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Recent studies in this clinic on the behavior of glucose solutions in the human stomach and duodenum[1] have indicated the need for a method to determine absorption from the isolated stomach. Experiments testing the absorption of drugs and foodstuffs by the gastric mucosa have been reported irregularly over a period of 60 years. In animals certain methods, some involving isolation of the stomach by obstructing ligatures or balloons[2, 3, 6] and others employing fistulas of the stomach and duodenum,[4, 5] have yielded clear-cut results, but they are not strictly applicable to normal man because each involves an operative procedure. In the intact human, because of the lack of a means of completely blocking the pylorus, the methods have been limited to the use of non-absorbable contrast materials for

* I wish to express my appreciation of the help given me by Dr. W. Osler Abbott, whose advice on tube construction and procedure in general has been invaluable, and by Mr. E. Freeman Hersey, who did the chemical analyses.

† Fellow in Gastro-Enterology and Assistant Instructor in Medicine, Medical School of the University of Pennsylvania.

1 Karr, W. G., Abbott, W. O., Hoffman, O. D., and Miller, T. G., to be published.

2 Tappeiner, H., *Z. f. Biol.*, 1880, **16**, 497.

3 v. Anrep, B., *Arch. f. Anat. u. Physiol.*, 1881, **2**, 504.

4 v. Mering, J., *Verhandl. des Kongresses der Innere Medizin*, 1893, **12**, 471.

5 London, R. S., and Polowzowa, W. W., *Z. f. physiol. Chem.*, 1908, **56**, 512.

6 Morrison, J. L., Shay, H., Ravdin, I. S., and Cahoon, R., in press.

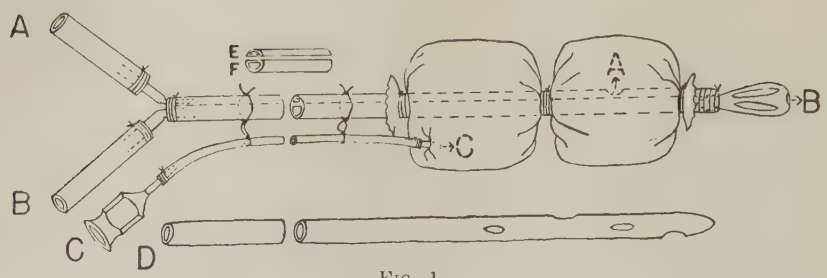

FIG. 1.

AB—Double lumen tube, length 115 cm, size 14 French. Lumen A is .07 x .2 cm, lumen B .3 x .2 cm. Distal balloon is 3.5 cm, proximal balloon 5 cm long, Rehfuss tip 1.8 cm long.

C—Single lumen 1/32" tubing attached to proximal balloon by a metal connection made from a No. 22 gauge needle. This tube is loosely bound to tube AB with the No. 5 silk thread that is used in the balloon bindings.

D—16 French Levin tube.

E and F—Bronze connections, 1.5 cm long, cast to fit the lumens of tube AB and to be inserted into them at binding sites.

comparison with test substances[7] and to attempts at pyloric closure by the use of "duodenal stimulants".[8] The latter technic in our hands was found to be unreliable. A mechanical method for pyloric occlusion has therefore been devised by which one may not only obtain satisfactory results, but which will unequivocally indicate technical errors when they occur.

Successful blockage of the pylorus in every attempt has been impossible because of the highly irritable state of that region: displacement of the occluding balloons and leakage past them tend to occur. Consequently it has been necessary to include procedures designed to detect failure of complete pyloric obstruction: fluoroscopy to show displacement of the balloons and an open tube in the duodenum to show leakage.

The apparatus used consists of 3 tubes (Fig. 1). A double-lumened tube of the Miller-Abbott type (AB) carries 2 balloons which obstruct the pylorus by holding it between them when they are inflated. The larger lumen (B), passing through the balloon system, communicates with a metal tip beyond and so allows for aspiration of duodenal contents during the experiment. The distal balloon is inflated via the smaller lumen (A) and the proximal one by a fine tube (C) running parallel to tube AB. A 16 French Levin tube with a catheter tip of the type familiar in clinical work serves to inject and to aspirate materials into and out of the stomach after the balloon system is in place.

Short segments of metal connecting tubing (E&F) are inserted

[7] Freund, I., and Steinhardt, P., *Deutsche med. Wchnschr.*, 1931, **57**, 1815.

[8] Shay, H., Gershon-Cohen, J., and Fels, S. S., *Ann. Int. Med.*, 1938, **11**, 1563.

into the lumens of the double-lumened tube at the sites where the ends of the balloons are to be bound and allow for tight binding without the possibility of constricting the lumens. The 2 balloons are made out of a single rubber condom. A 3-lumened tube may be used to replace the combination of the double-lumened and the small tubes. The present arrangement, however, has been used because of its greater pliability.

The experiment is done under fluoroscopic guidance. The fasting subject swallows the balloon system first, and the metal tip is allowed to slip into the duodenum. Tube D is then swallowed until its tip has reached the most dependent part of the stomach. The stomach is emptied of its fasting contents. Five cc of air are then injected into the distal balloon, and this set of tubes is withdrawn, leaving tube D undisturbed, until the partially inflated balloon is on the point of falling back into the stomach. An additional 25 to 45 cc of air, the amount in each instance depending on the activity of the

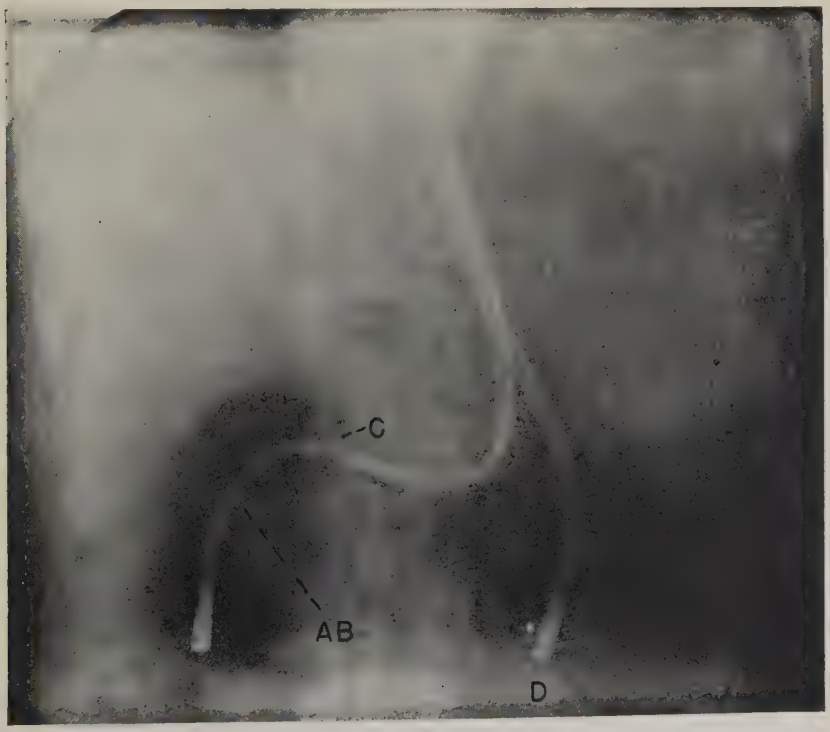

FIG. 2.

An early experiment indicating the fluoroscopic appearance of the apparatus in place. This apparatus is identical with that in Fig. 1 except for the character of the tips of the tubes.

duodenum and the amount of pressure resisting the injection, are introduced rapidly into the distal balloon. The subject is then instructed to put moderate traction on the 2-lumened tube with his hand in order to prevent its passage along the duodenum. The experimenter injects 50 cc of air into the proximal balloon while the tube is thus held. This should leave the balloons athwart the pylorus, one in the duodenal cap and the other in the antrum of the stomach. In some of the experiments it has been suspected that the proximal balloon was also in the duodenal cap. In either event it is obvious from their position as seen under the fluoroscope that none of the duodenal mucosa can be exposed for absorption. Fig. 2 shows the fluoroscopic appearance of the apparatus in place.

With the balloons thus in place test substances may be introduced into the stomach through tube D and later aspirated at the end of a predetermined period via the same tube. Subsequent lavage of the stomach with about 800 cc of water in divided portions thoroughly rids it of all traces of remaining test substance. As previously stated continuous aspiration through lumen B must be maintained throughout the experiment to allow for detection of any escape of gastric material into the duodenum.

For the purpose of determining the efficiency of the technic, glucose has been used as the substance to be tested. Of numerous experiments attempted the proportion of successes to failures has so far been about 3 to 5. The causes for failure in the unsuccessful experiments have been slipping of the balloons, vomiting of the test substance, faulty construction of the apparatus and escape of glucose past the balloons. These were all promptly recognized and the experiments discarded. Two sample successful experiments are tabulated in Table I.

That the glucose was lost in considerable amounts from the concentrated solution but in negligible quantities from the dilute solution is to be noted. A discussion of the significance of this fact is not within the scope of this paper and will be reserved for a later report.

TABLE I.
Gastric Absorption of Glucose.

	Amount injected			Test period, min.*	Duodenal specimen		Amount aspirated			
							Gastric specimen		Gastric wash	
Subject	cc	g	%		cc	g	cc	g	cc	g
H	250	12.5	5	34	17.5	0	274	11.23	617	.72
P	249	149.4	60	35.5	11	0	385	118.6	997	11.07

*Calculated from onset of injection to onset of wash.

Summary. A technic for the study of gastric absorption is presented by which the pyloric opening can be completely blocked without operation or disturbance of blood supply in human subjects that are in every way normal. Methods of detecting immediately failure of the blocking device insure against false surmise in the interpretation of results.

10650

Histological Changes in Skeletal Musculature of Paralyzed Suckling Young of E-Low Rats.*

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Evans and Burr[1] first described the partial or complete paralysis affecting a high percentage of suckling young of vitamin E-deficient rats. Olcott[2] found that a degeneration of the cross striated musculature occurs in such paralyzed rats not unlike the nutritional muscular dystrophy earlier observed in herbivores by Goettsch and Pappenheimer,[3] Woodward and McCay,[4] Madsen, McCay and Maynard,[5] Victor,[6] and Morgulis and Spencer.[7] Lipschutz,[8] although not studying the musculature, reported that suckling E-deficient rats had definite lesions in the vestibular nuclei and their connections, and in the extra pyramidal tracts, proprioceptive tracts, and ventral horn cells of the cord. Olcott[2] observed "no abnormalities in the nerves,

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1 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

2 Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

3 Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

4 Woodward, J. C., and McCay, C. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 241.

5 Madsen, L. T., McCay, C. M., and Maynard, L. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1434.

6 Victor, J., *Am. J. Physiol.*, 1934, **108**, 229.

7 Morgulis, S., and Spencer, H. C., *J. Nutrition*, 1936, **11**, 573.

8 Lipschutz, D., *Revue Neurologique*, 1936, **65**, 221.

cord, or brain." An investigation of all neurological lesions in vitamin E-deficiency is in progress in this laboratory.

Materials and Procedure: A total of 151 suckling rats were used in this study. All were born of young E-deficient mothers of proved sterility which had received, on the day of the second positive mating, one gram of a standardized wheat germ oil, a quantity little more than sufficient to insure a single normal gestation. Each litter was divided into experimental and control groups. The 81 experimental E-low rats were kept with their mothers held on E-free diet 808.†

Seventy control littermates were treated by 2 methods, both with equal success. Wheat germ oil was administered to one control group 6 times weekly by dropper or stomach tube after day 5 or day 10 until weaning. The second control group was placed with foster E-free mothers which were given a dose of 2 g of wheat germ oil immediately following parturition.

The 81 suckling young of the untreated mothers presented the following clinical pictures:

(1) 31 showed no visible signs of paralysis.

(2) 50 showed some degree of paralysis—varying in severity from a slight gait impairment to a complete loss of function of the hind limbs and a marked reduction in forelimb action.

Claw flexion of the foretoes was frequently observed in these animals. Such rats moved only by the action of the forelimbs, with the hind extremities passively extended. They found it extremely difficult to right themselves when placed on their side or back and vigorous tail movement was the principal factor in enabling them to recover their equilibrium. Another consistent observation with these animals was the presence of a thin corneal film. The development of this syndrome may be a gradual process extending over a period of from 2 to 5 days, or it may have a sudden onset, being full flowered in the morning, when not apparent the preceding evening. Young rats that develop the paralysis may (1) die suddenly, (2) linger for a maximum period of 8 to 10 days and then die, or (3) recover spontaneously without the administration of vitamin E to the diet.

† Diet 808:

Casein (commercial)	27
Cornstarch (cooked)	30
Lard	22
Cod liver oil	2
Brewers' yeast	15
Salts No. 185	4

(With the exception of the cod liver oil, all ingredients of the diet were allowed to stand at room temperature for 2 weeks. The resulting rancidity of the lard destroyed any vitamin E in the diet. Cod liver oil was added just before feeding.)

This experiment was planned to correlate the clinical conditions of these animals with the observed histological changes.

Upon autopsy at 3 weeks of age, the gastrocnemius and soleus muscles were removed, spread on a strip of cork and fixed immediately in Zenker-formol or Susa fixative. Other muscles such as the sternohyoid, deltoid and trapezius were in some instances removed and treated as the above. The tissues were embedded by the rapid nitrocellulose method of Koneff and Lyons,[9] sectioned at 6 to 8 micra and stained with Harris hematoxylin-eosin, iron hematoxylin-aniline blue,[10] and iron hematoxylin-aniline-blue-methyl green.‡

Experimental. Degeneration was demonstrable in the skeletal muscles of all paralyzed suckling young of E-deficient rats. The progress of the malady may be divided into 2 phases—(1) a degeneration stage and (2) a regeneration stage.

The degenerative phase manifests itself in 2 pathological processes—firstly, a hyaline or Zenker degeneration of the fibers themselves, and, secondly, the infiltration of white blood cells and connective tissue elements with reactive multiplication of muscle nuclei.

Figure 1 shows the histological picture of the normal skeletal muscle.

The first stage of degeneration can often be demonstrated histologically before the animal shows any obvious signs of paralysis. The lesions are confined to separate or small groups of fibers, healthy normal muscle tissue being interspersed (Fig. 2). The lesion manifests itself as a waxy, hyaline or Zenker degeneration of the individual muscle fibers. In the necrosis of the fiber, the contractile substance becomes coagulated and breaks up into swollen homogeneous hyaline segments. These masses may attain a diameter double that of the normal fiber. As the degeneration progresses the swollen segments (1) lose their hyalinization and become granular, (2) become reduced in size, (3) and finally become debris in the intrasarcolemmal space. The muscle nuclei also undergo change. They are crowded against the sarcolemma of the swollen fiber segments and are pyknotic.

As the degeneration progresses an increasing number of the muscle fibers are affected (Fig. 3). The rapid loss of cross and longitudinal striations is striking. The fibers are non-continuous, segmented, amorphous masses. The cytoplasm of the affected fibers stains darker with eosin. The beginning invasion of phagocytic cells

9 Koneff, A. A., and Lyons, W. R., *Stain Technology*, 1937, **12**, No. 2.

10 Koneff, A. A., *Anat. Record*, 1936, **66**, 173.

‡ Developed by Dr. A. A. Koneff of the Division of Anatomy, procedure as yet unpublished.

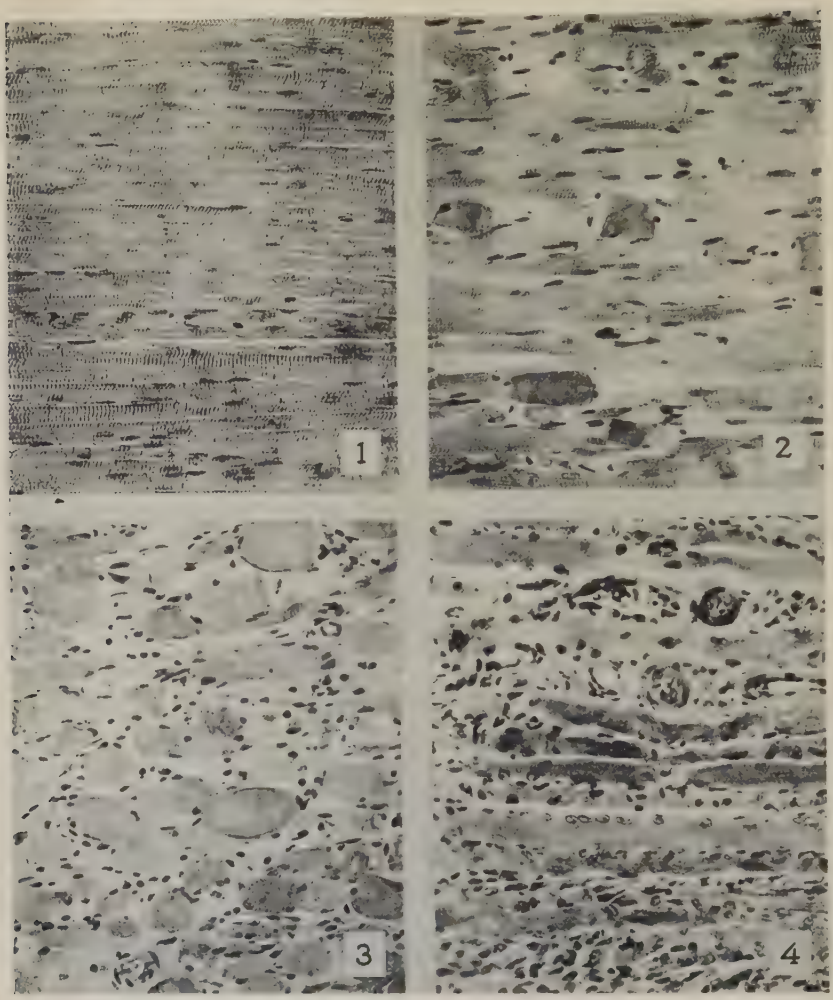

FIG. 1. Section of gastrocnemius muscle from a normal 21-day-old rat.
FIG. 2. Section of gastrocnemius muscle from a 21-day-old vitamin E-low rat with no obvious paralysis.
FIG. 3. Section of gastrocnemius muscle from a 22-day-old vitamin E-low rat with marked paralyses.
FIG. 4. Section of gastrocnemius muscle of a 24-day-old vitamin E-low paralyzed rat showing leucocyte infiltration and muscle nuclei multiplication stage.
All slides stained with hematoxylin and eosin. × 243.

is now noticeable. These histological changes are found in animals showing marked locomotive impairment.

When clinical signs of definite paralysis occur, such as dragging of the hind extremities, the second stage can be histologically demonstrated. This phase is characterized by the fact that there has been

an extensive infiltration of leucocytes and rows of muscle nuclei indicate the position of preëxisting fibers (Fig. 4). Some of the original muscle nuclei, displaced and distorted by the swelling of the fiber, are also found crowded on the sarcolemma. Later they undergo complete karyorrhexis and may disappear. The collapsed fibers often contain only granular debris and newly formed nuclei.

In the succeeding stage of this dystrophy, characterized clinically by an improvement in locomotion and lessening of the paralysis, there is already histologically a regeneration of part of the muscle fibers. Within an area filled with infiltrated leucocytes, connective tissue elements and debris, newly formed isolated fibers appear. In accordance with the description given by Forbus[11] of regenerating skeletal muscle, these fibers always arise in connection with the rows of muscle nuclei. Subsequently myo-fibrils develop between the nuclei and regenerated fibers with well defined cross and longitudinal striations appear. The nuclei stain lightly with hematoxylin and are at first centrally placed in the fiber.

Summary. The skeletal musculature of paralyzed young of E-low rats invariably shows a progressive degeneration which is correlated with the severity of the paralysis. In those cases where, as described in the preceding paper, a spontaneous recovery from the paralysis has occurred, a regeneration of the musculature can be observed to have taken place.

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Experimental Production of Arteriolonecrosis and Medionecrosis of Arteries by Means of Tyramine Injections.

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(Introduced by W. L. Holman.)

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It is the opinion of many workers that certain forms of hypertension are produced by means of a pressor substance elaborated in ischemic renal tissue. Wolf and Heinsen[1] have expressed the belief that tyramine is in fact the pressor substance responsible for the development of hypertension in animals rendered hypertensive by

11 Forbus, W. D., *Arch. Path.*, 1926, **2**, 318.

1 Wolf, H. J., and Heinsen, H. A., *Arch. f. exp. Path. u. Pharmakol.*, 1935, **179**, 15.

the establishment of renal ischemia. Some support is given to this hypothesis by the derivation of a pressor principle having many of the properties of tyramine from the digested saline extract of renal cortex.[2] Whether or not tyramine is concerned in human hypertension, it seemed of value to determine whether systemic injection of tyramine can produce vascular lesions since it is generally acknowledged that the arteriolar lesions found in association with arterial hypertension in man are of fundamental importance in relation to the disease.

In suitably controlled experiments lasting from 1 to 106 days, fresh 1% solutions of tyramine (Hoffman-La Roche) in sterile normal saline were injected daily into the ear veins of 10 rabbits under 6 months of age maintained on an adequate diet. In preliminary experiments initial doses of over 100 mg of tyramine had proved fatal so that a daily dose of from 50 to 100 mg was adopted for all except one rabbit in which the dose was gradually increased up to 190 mg without fatal results, indicating the development of a degree of tolerance. In all animals pupillary dilatation and hyperpnoea lasting up to 45 minutes followed the injections of tyramine. In 7 rabbits death eventually occurred in convulsions after an injection. The other experimental animals and the controls were killed with ether. Complete autopsies were done and thorough histological studies carried out in each case.

Although lesions of the arterial tree were not produced with regularity, 6 of the 10 rabbits receiving tyramine showed significant vascular lesions. Arteriolar lesions were present in 4 animals. In 2 of these and in 2 additional rabbits medionecrosis was seen in the aorta or in the large arteries, particularly the renals, or in both. In the aorta and large arteries pale anuclear areas of necrosis were present over large segments of their walls, spreading out from the center of the media to occupy the greater part of its thickness. Weigert stains revealed intact elastic fibers in these areas; calcification was lacking. These lesions differed in several respects from the familiar spontaneous medial lesions of the rabbit's aorta.

Arteriolar lesions, in the 4 animals showing them, were encountered in brain, kidney and heart, but all 3 organs were not always involved, nor were all the arterioles affected in any one organ. Thus, careful search was necessary to determine the presence of arteriolar lesions in the organs least affected. The slightest alterations were pyknosis, or swelling and fading of medial nuclei, followed by frag-

2 Williams, J. R., Jr., Harrison, T. R., and Mason, M. F., *Am. J. M. Sc.*, 1938, **195**, 339.

mentation or entire disappearance of medial nuclei and of the elastica. The cells of the lining endothelium were intact but swollen. In some cases there was thickening of the vessel walls by the deposition of a deeply eosinophilic substance which spread apart the partially necrotic cells of the media. The most advanced changes consisted of complete necrosis of arterioles with glassy, homogeneous, hyaline swelling of their walls, accompanied by a more or less marked accumulation of pink-staining, homogeneous material in the adventitial tissues. In one animal in which extensive cerebral arteriolonecrosis was found, massive cerebral hemorrhage had resulted in death. It was not possible to establish a relationship between the occurrence or extent of the vascular lesions and the dosage of tyramine or the duration of the experiment. We are, therefore, endeavoring to discover what factors determine the development of vascular lesions.

Some of the experimental arteriolar lesions resembled very closely the arteriolonecrosis commonly associated with malignant hypertension in man, as well as the arteriolonecrosis occurring in dogs in which malignant hypertension has been produced by the establishment of severe renal ischemia (Goldblatt[3]). Although Rich and Duff[4] have produced local necrosis and hyalinization of vessels by the subcutaneous injection of trypsin, widespread hyaline arteriolonecrosis has not hitherto been produced, so far as we are aware, by the systemic injection of any substance.

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3 Goldblatt, H., *J. Exp. Med.*, 1938, **67**, 809.

4 Rich, A. R., and Duff, G. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 470, and *Bull. Johns Hopkins Hosp.*, 1937, **61**, 63.